Diabetic retinopathy: Molecular mechanisms, present regime of treatment and future perspectives

K. N. Sulochana[†], S. Ramakrishnan[†,*], M. Rajesh[†], K. Coral[†] and S. S. Badrinath[#]

[†]Biochemistry Research Department and [#]Vitreoretinal Department, Vision Research Foundation, Medical Research Foundation, Sankara Nethralaya, 18, College Road, Chennai 600 006, India

Diabetes mellitus (DM) has become a common disease with serious complications. One of the most devastating microvascular complications of DM is diabetic retinopathy (DR). Over the last decade considerable progress has been made in the understanding of the pathogenesis of DR, and several factors have been implicated in its pathogenesis. These include non-enzymatic glycation, glycoxidation, accumulation of advanced glycation end-products, free radical mediated protein damage, up-regulation of matrix metalloproteinases, elaboration of growth factors and secretion of adhesion molecules in the vascular endothelium. The exact molecules involved and the precise mechanism of their interaction in the development of retinopathy are presented here. Also discussed are the conventional and molecular methods of treatment for DR and the growing need for the development of a novel, pharmacological therapeutic regime.

DIABETES mellitus (DM) represents a major medical problem affecting millions of people all over the world. The two distinct forms of DM are type 1, juvenile or insulin-dependent diabetes mellitus (IDDM) and type 2, adult onset or non insulin-dependent diabetes mellitus (NIDDM)[1]. Type 1 patients run a higher risk of severe ocular complications. Approximately 25% of patients with type 1 DM have been shown to be affected with retinopathy, with the incidence increasing to 60% after 5 years and 80% after 10 to 15 years of affliction[2]. However since there are more adult onset cases than juvenile ones, type 2 DM accounts for a higher proportion of patients with visual impairment. Despite a significant improvement over the past two decades in our understanding and treatment of the ocular complications of DM, many aspects about this subject need to be elucidated, because the management and treatment of diabetic retinopathy (DR) still remains one of the important challenges of the medical profession.

*For correspondence. (e-mail: drsr@sankaranethralaya.org)

The early stages of DR are characterized by histopathological changes which include loss of pericytes, basement membrane thickening, haemodynamic alterations (changes in retinal blood flow and areas of capillary non-perfusion), vascular abnormalities (micro aneurysms, intraretinal microvascular abnormalities and venous bleeding) and reduced vascular integrity[3,4]. The later stages of DR are characterized by complications, which include visual impairment, primarily due to macular edema and proliferative diabetic retinopathy (PDR, Figure 1). In PDR newly developing vessels commonly arise from the retina and optic disc, but many can be found also on the iris and the trabecular meshwork. These new vessels are fragile and prone to rupture, resulting in vitreous haemorrhage, and subsequent detachment of the retina. If vessels proliferate on the iris or trabecular meshwork, the normal outflow of the aqueous fluid may be impaired leading to neovascular glaucoma and permanent optic nerve damage[5].

Hyperglycemia and DR

Although prolonged hyperglycemia has been thought to play a major role in causing the complications of diabetes, there has been a controversy on this issue. Until recently, all studies on the relationship between levels of glycemia and the complications of diabetes have been retrospective[6], except a diabetic control and complication trial (DCCT) which has been prospective[7]. Most investigators believe that excellent blood glucose control decreases the risk of retinopathy. However there have been important dissentions based largely on the observation of severe complications in some diabetic individuals whose blood sugar control was found to be good, and a few with no complications even when controlled poorly[8–10]. Besides, there have been a few controversial reports of non-diabetic individuals from families with several diabetic members, exhibiting retinopathy indistinguishable from DR[11]. Reports of large-scale studies (some of which assessed long-term blood glucose control by glycosylated haemoglobin determi-

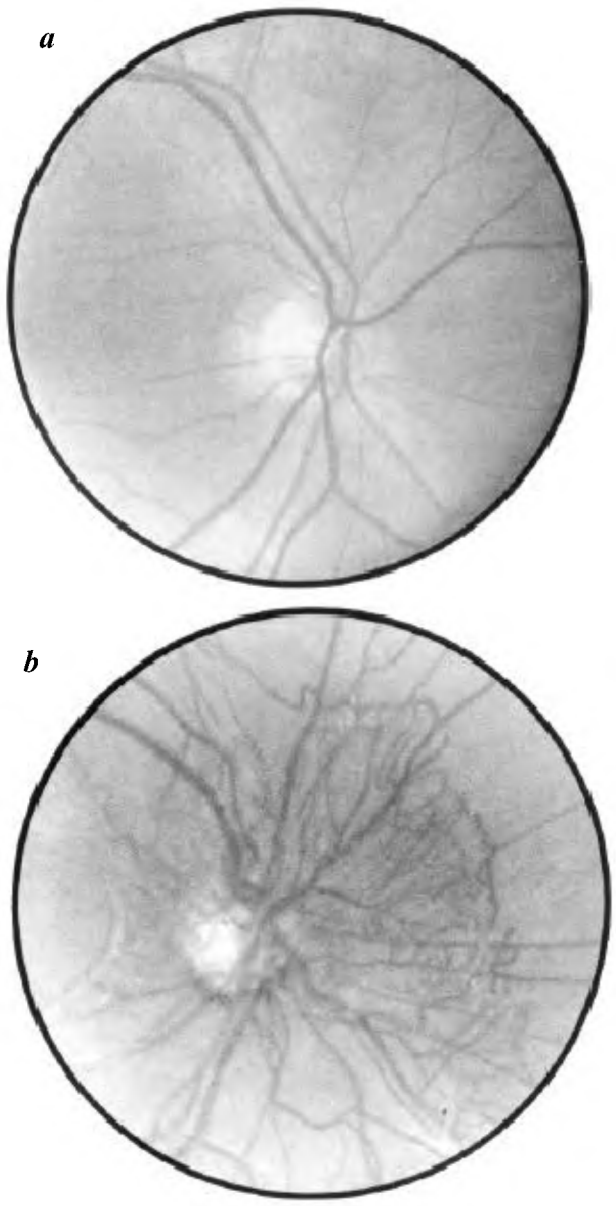

Figure 1. ***a***, Fundus of a normal eye; ***b***, Fundus showing extensive neovascularization from the optic disc in a case of proliferative diabetic retinopathy.

nations and compared this with the levels of retinopathy assessed by fundus photography[12,13]) on this relationship are available. These studies provided evidence that severity of retinopathy was associated with poorer metabolic control, demonstrated by elevated HbA_1c, but suffered from shortcomings; they were not prospective because the subjects had been randomly allocated to metabolically 'tight control' and 'loose control' groups. The results therefore gave rise to the possibility that glycemia may not be directly related to retinopathy or other diabetic complications[12,13].

Polyol pathway and DR

The polyol pathway (sorbitol pathway) has been hitherto largely held responsible for DR. Glucose on reduction by NADPH gives rise to sorbitol, by the action of aldose reductase; the sorbitol so formed is converted to fructose in the presence of sorbitol dehydrogenase. Sorbitol does not diffuse through cell membranes easily and accumulates, causing osmotic stress[14]. Simultaneously, the myoinositol level falls, which in turn affects the Na^+K^+ ATPase pump, leading to hydration[15]. The cells of other tissues in the body that develop complications on account of diabetes have been shown to have more activity of the glycolytic pathways, the citric acid cycle and the pentose shunt than in most part of eye. Due to prolonged hyperglycemia, these cells have been found to develop elevated levels of sorbitol, but not sufficiently high to cause osmotic stress, indicating that sorbitol accumulation is alone not responsible for the development of retinopathy. Moreover the implications of aldose reductase in development of DR is a paradox, due to the fact that this enzyme does not function at physiological concentrations as it has a very high K_m (Michele's Menton constant) for glucose, 50 times more than the concentration of 10–15 mM in diabetes[15]. Hence use of aldose reductase inhibitor as a treatment for DR is questionable.

Role of nonenzymic glycation in DR

Glucose and other hexoses react with most of the tissue/cellular proteins by combining with free ε-NH_2 group of lysine residues to form Schiff's base which further undergoes a series of changes, to yield stable fluorescent adducts termed as Maillard products (Figure 2) or advanced glycation end products (AGEs)[16]. AGEs are a group of fluorescent compounds chemically distinct from each other. AGE products which have been characterized, well understood and implicated in various pathological conditions are carboxy methyl lysine (CML), fructosyl furanoyl imidazole (FFI), pentosidine and methly glyoxal (MG)[17,18]. Once the protein gets glycated, it undergoes further modification and forms protein–protein cross-link, by the oxidation of SH groups in the proteins. Collagen–collagen cross-linking may be particularly responsible for the basement membrane thickening which is considered to be one of the common histopathological changes observed during development of DR, diabetic neuropathy and diabetic nephropathy[16]. Use of antiglycating substances such as aminoguanidine has been reported to decrease protein cross-linking[19].

The amount of AGEs found on various body proteins is far less than would have been anticipated. The reason for this is macrophages have a unique high-affinity

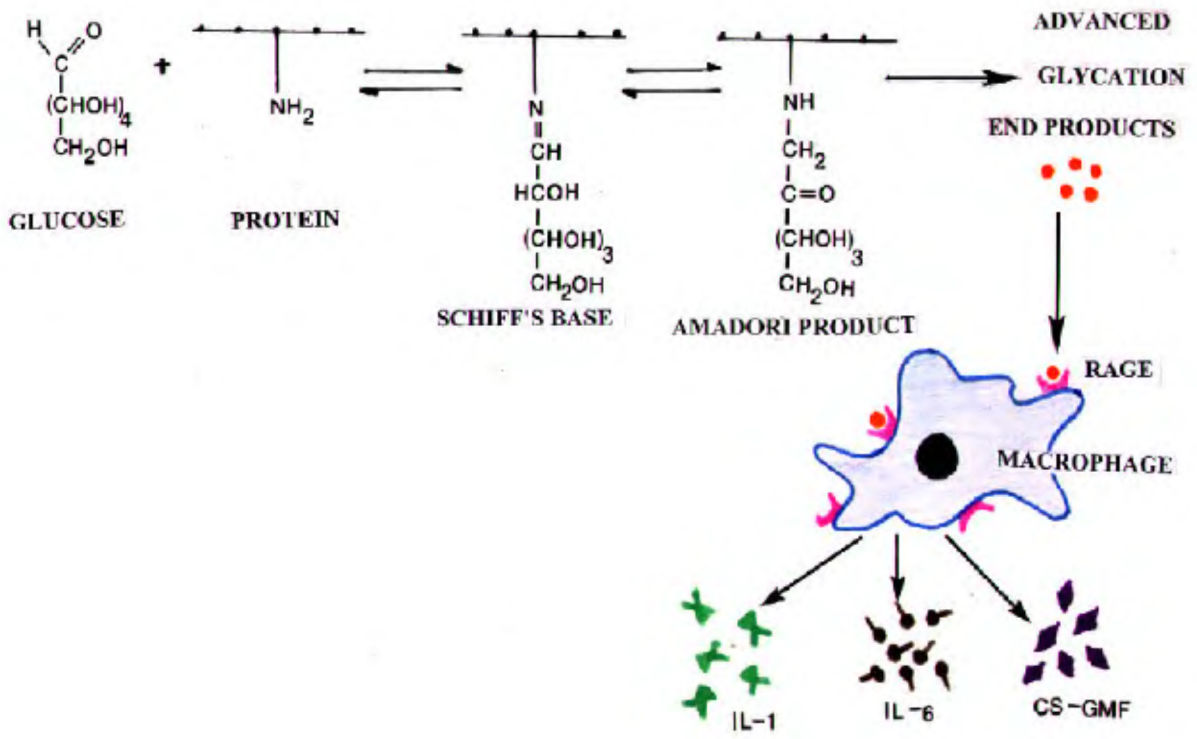

Figure 2. Pathways of AGEs formation. Glucose and other reducing sugars condense nonenzymatically with ε-NH_2 group of proteins to yield a Schiff's base which undergoes a series of rearrangements to yield a group of stable adducts called advanced glycation end products (•, AGE). When AGEs bind to their receptors (RAGE) on macrophages, they are internalized and degraded. During this process macrophages release certain chemokines interleukin IL1 and IL6, and colony-stimulating granulocyte-monocyte factor (CS-GMF).

receptor (RAGE) that mediates the uptake and degradation of AGE proteins[20]. The net tissue accumulation of AGE protein reflects equilibrium between two opposing processes, i.e. glucose-mediated rate of formation and macrophage-mediated rate of removal. In diabetes it is possible that this removal system is not completely efficient because, AGEs continue to accumulate over an individual's entire life span as a function of chronological age and plasma glucose level. In the diabetic condition, the accelerated rate of accumulation of AGE proteins may be beyond the ability of the body to remove these products[19]. This has been evidenced with the increasing number of reports associating diabetic complications with accumulation of AGEs in diabetic tissues[19]. Increased accumulation of AGEs has been reported in epiretinal membranes (ERM) surgically excised from patients with DR, using immunohistochemical technique[21–25].

Recently RAGE has been localized in retinal endothelial cells, RPE and pericytes[20,26]. AGE molecules upon binding to their receptors, exert diverse actions on target cells. Unlike in macrophages, the effect of binding of AGE to RAGE in these cells is different. Binding of AGE to RAGE in pericytes causes the death of the pericytes, while in RPE and endothelial cells, it promotes their migration and proliferation. Retinal microcapillaries are lined by endothelial cells and pericytes[27].

Loss of pericytes has been reported to be the hallmark of DR[28]. Why focus on pericyte loss/death? The answer lies in the fact that pericyte controls the integrity of the endothelium, endothelial cell proliferation and maintenance of blood retinal barrier by maintaining endothelial cell/pericyte ratio as 1:1. The mechanism suggested for this action is that, pericyte by synthesizing transforming growth factor (TGFβ) has an inhibitory effect on endothelial cell migration and proliferation[29,30]. Probably pericytes may synthesize and secrete other negative modulators of neovascularization, which needs to be studied. Some of the positive and negative modulators of neovascularization are listed in Table 1. It has been reported earlier that in pericyte–endothelial coculture systems, the pericytes can not only regulate cell proliferation and growth, but also preserve the prostacyclin-producing ability and capacity to protect against free radical-induced injury to endothelial cells[26]. Pericytes are contractile elements actively maintaining blood retinal barrier by exerting a constant tonus on the capillary tube or providing a localized increase in tonus, shifting the flow of blood from one part of the capillary bed to another or both. The capillary pericytes have

Table 1. Positive and negative modulators of neovascularization

Positive modulator		Negative modulator	
Factor	Source	Factor	Source
Growth factor	RPE, endothelial cells, macrophage and platelets	TIMP (1–4)[41]	Tissue
Angiogenin[81]		Angiostatin[92]	ECM
Angiotropin[82]		Endostatin[93]	Endothelium
bFGF[83]		Thrombospondin[94]	Platelets, RPE and endothelial cells
PD-EGF[84]			
TGFα and β[85]			
TNFα[86]			
VEGF[87]			
IGF[88]			
HGF[42]			
Proteases			
MMP[43]	Wide distribution in ocular tissue		
Urokinase[89]			
Adhesion molecules			
Integrin[90]	ECM, platelets and endothelial cells		
ICAM[91]			
VCAM[91]			
PECAM[91]			

been shown to contain abundant actin fibrils oriented in parallel arrays to facilitate their contraction[31]. Structural and functional loss of these actin filaments may contribute to pericyte dysfunction. Actin, like any other body protein could be prone to glycation. Glycation of actin in turn will lead to dysfunction of pericytes, which may also contribute to pathogenesis in DR. However, the precise molecular cascade behind the dysfunction or death of pericyte remains elusive and poorly understood[32]. Although a recent report by Yamagishi *et al.*[33] points out that AGE binding to RAGE in pericytes exerts selective toxicity resulting in their death.

Unlike in pericytes, when AGE binds to endothelial RAGE, it induces a plethora of events including gene expression for a variety of molecules, viz. growth factors such as vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF)[34,35], transcription factors ($NF_{\kappa}B$, SP1 and STAT 1)[32], activation of proteases such as matrix metalloproteinases (MMP), capsases and calpines[24], and the synthesis of adhesion molecules like vascular endothelial cell adhesion molecule (VECAM), E-SELECTIN, platelet endothelial cell adhesion molecule (PECAM) and intercellular adhesion molecule (ICAM)[36]. All these events result in the disruption of the cellular homeostasis in DM. The adhesion molecules stimulate cell–cell adhesion and cell–extracellular matrix (ECM) interaction. This process recruits macrophages to the local site (vessel wall), setting the stage for diffused and accelerated artherogenesis by elaborating cytokines and growth factors to activate the cascades. These growth factors aid in angiogenesis, thrombogenesis and artherogenesis (Figure 3).

The important stages involved in the angiogenic process are invasion, migration and proliferation of microvascular endothelial cells through the capillary basement membrane and their seepage to adjacent ECM, leading to the growth of new microvessels. This invasion is coupled with the production and activation of specific extracellular protease, viz. serine proteinase–urokinase and enzymes of matrix metalloproteinases (MMPs) family. Blocking the binding of AGEs to RAGE using synthetic chimerical RAGE will be of therapeutic value.

Role of MMP in DR

MMP belongs to a family of enzymes, contributing to both normal and pathological tissue remodelling[37]. MMPs play a key role in the migration of normal and malignant cells through the body (Figure 4). They also act as regulatory molecules, both by functioning in enzyme cascades and by processing matrix proteins, cytokines[38], growth factors and adhesion molecules to generate peptide fragments with enhanced or reduced biological effects. So far 24 MMPs have been characterized. They are Zn-containing endopeptidases, and require Ca^{2+} for their action. They have been classified into four groups according to their substrate specificity, viz. Collagenases, Stromelysin, Matrilysin and Gelatinases. Collagenases cleave interstitial collagen types I,

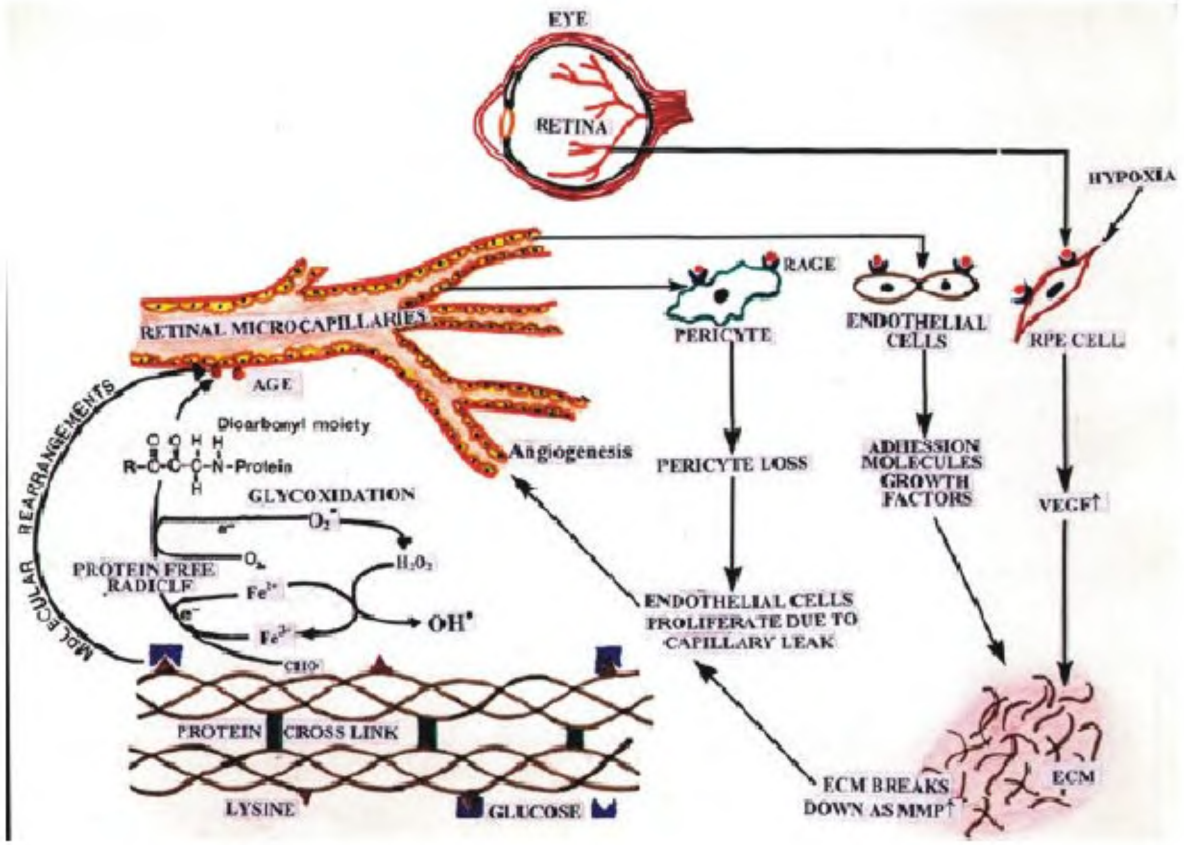

Figure 3. Molecular mechanism in the pathogenesis of DR. Glycation leads to protein–protein crosslink, which further undergoes molecular rearrangements to form AGE. Besides, available hydrogen peroxide with the aid of metal ions (Fe^{+3}) attacks the carbohydrate moiety (CHO) in the protein to form dicarbonly derivatives, leading to AGE formation. The AGEs thus formed in these ways, bind to RAGE on the endothelial cells, pericytes and RPE cells and perturb a wide range of cellular events and vascular homeostasis, leading to retinal neovascularization in DR.

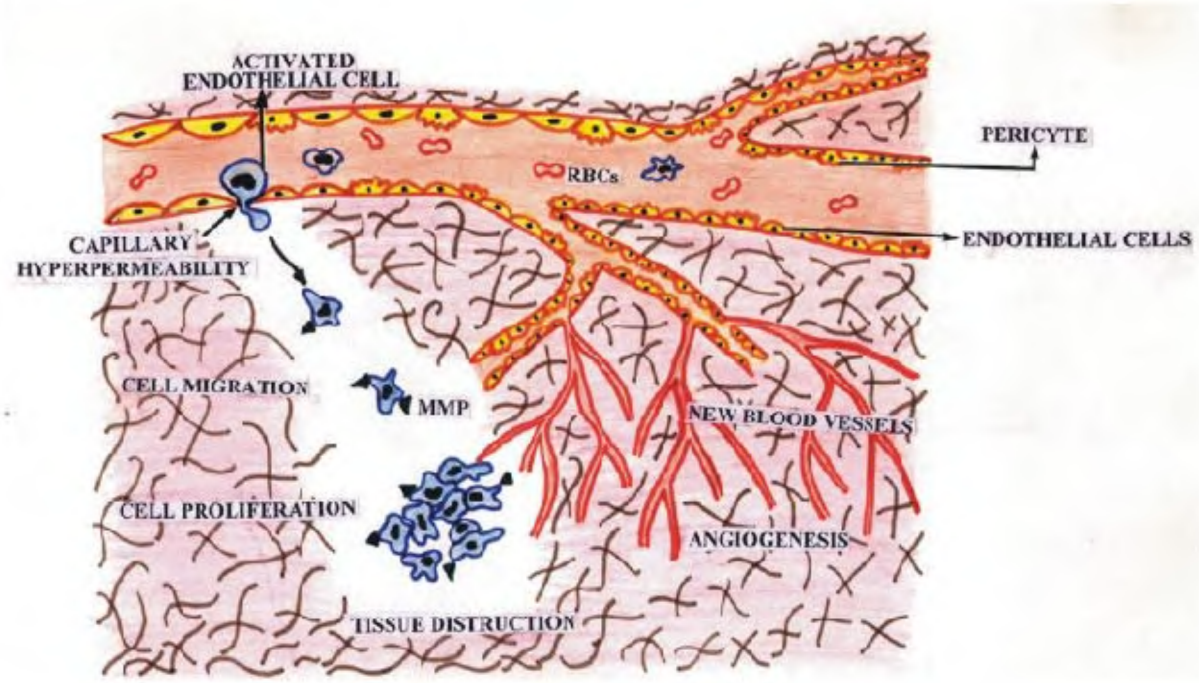

Figure 4. Role of MMP in DR. Once AGE binds to RAGE on the endothelial cells, it activates the cells to express certain adhesion molecules. These activated endothelial cells adhere to the extracellular matrix of vascular bed and migrate and proliferate with the simultaneous production of MMP and growth factors. These events result in colonization of activated endothelial cells, which ultimately results in the formation of new vessels.

II and III. Stromelysin and Matrilysin cleave all ECM components. Gelatinases specifically cleave gelatin, fibronectin, laminin, elastin, MMP 2 and 9 and collagen IV, V, VI and X. These enzymes are produced by a variety of cell types like endothelial cells, macrophages, fibroblasts, inflammatory cells and malignant cells. They play a pivotal role in migration and proliferation of endothelial cells during the angiogenesis process occurring in DR (Figure 4)[39]. MMP 2 & 9 have been associated with DR, a fact supported by their localization in epiretinal membranes. Increased activity of MMP 2 & 9 has been revealed by MMP zymography[40]. Endogenous inhibitor of MMP, i.e. tissue inhibitor of MMP (TIMP) has been shown to feedback, inhibit or down-regulate the activity of MMP. Currently four TIMP members have been characterized (TIMP 1–4)[41]. At any given point of time the equilibrium between MMP and TIMP molecules maintains the normal homeostatic process in the intra-vascular milieu. Recently it has been found that MMPs generate two other endogenous inhibitors of angiogenesis, namely angiostatin and endostatin[42,43]. More efforts should be directed towards identifying the specific MMP that mediates the angiogenic response in diabetic retina, so that a specific MMP inhibitor can be used for the therapeutic intervention. General MMP inhibitors such as thalidomide, marimastat, etc. (Table 2) will not be of much use.

Role of growth factors in DR

Growth factors can be defined as protein hormones secreted by an organ, a tissue or a cell, having a growth-stimulating role where they are produced or in the target cell or tissue. Although many growth factors having potential angiogenic property have been identified, only five of them have so far been implicated in DR. They are basic fibroblast growth factor (bFGF), insulin like growth factor (IGF), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF) and hepatocyte growth factor (HGF). bFGF a heparin-binding protein is associated with extracellular matrix[44]. It stimulates endothelial cell proliferation, migration and neovascularization in chick chorioallantoic membrane and cornea models[44,45]. However it is not secreted actively from cells because it lacks signal peptide for secretion[46–48]. Although bFGF is present in the retina, no relationship with neovascularization has been established in ocular fluids collected from patients with PDR[49]. IGF stimulates migration and proliferation of retinal cells. However the neovascularization effect has not been demonstrated at physiological levels[50]. IGF-1 has been found to be elevated in vitreous and serum of patients with PDR, whereas IGF-2 is not elevated[51]. In diabetes, the vitreous concentrations of both IGF-1 and IGF-2 were correlated with serum concentrations.

Table 2. Molecular intervention of diabetic retinopathy

Target	Agent	Mechanism
Glucose-lowering agents	OHA and insulin	Insulin potentiating agents
	Ascorbose[70]	α glucosidase inhibitor
Enzyme		
Aldose reductase	Sorbinil[95]	Inhibits aldose reductase
ACE	Captopril[96]	ACE inhibitor
MMP	Lovastatin[97], Batimastat[97], Marimastat[97], Psorvastat[97], Neovastat[97], Neoretha[97] and Thalidomide[97]	Inhibits MMP
Anti non enzymatic glycosylation agents	Aminoguanidine[98]	Inhibits AGE formation
	Amino acids[76–79]	Mitigates glycation, improves insulin sensitivity
	Ibubrufen, paracitamol and aspirin[99]	Antiglycating agents
Anti growth factor	VEGFR chromic protein[100]	Blocks the VEGF receptor on endothelial cell
	Anti-VEGF mab[101]	Monoclonal antibody
	Antisence oligonucleotide[102]	Inhibits mRNA translation
	Suramin[103]	GF antagonist
	Pentosan poly sulphate[104]	GF antagonist
	Octreotide[105]	GF antagonist
Signal transduction	Flavopuridol[106]	PKC inhibitor
Oxidant stress	Vitamins E and C	Prevents lipid peroxidation and scavenge free radicals[107,108]
Minerals	Zn, Se	Decreases MMP cofactor for antioxidant enzymes like GP_x, SOD activity[109]

Levels of IGF-1 and IGF-2 in the vitreous of patients with PDR have been reportedly elevated in a large population-based study[52]. A comprehensive study of more than 1000 patients in a well-characterized population from Wisconsin, USA evaluated serum IGF-1 levels and retinopathy status at baseline and after a six-year follow-up[53]. The diabetic group was further allocated into older onset and younger onset patients, and by their use of insulin. The incidence of retinopathy was not higher in people with higher IGF-1 levels, and it was concluded that serum IGF-1 was not associated with progression of DR in any of the subgroups studied. Thus, it is also unlikely that IGF-1 is the predominant mediator of intraocular neovascularization in DM. PDGF have been reported to be elevated in cases of proliferative viteroretinopathy (PVR) and PDR[54]. And it is also up-regulated by AGE in cultured retinal pigment epithelial (RPE) cells[35]. Recently HGF has been found to be expressed in RPE[55]. Robins and his coworkers[54], observed elevated levels of HGF in ocular fluids and compared them with serum HGF levels from patients with type 2 DM. The fact that they have observed higher levels of HGF in vitreous suggests that this factor is synthesized locally and elicits its action in the local site where it is produced. This gives further strength to the proposition that HGF might have a potential role in retinopathy. The presence of hypoxia-inducible growth factor – VEGF in RPE cells has been demonstrated by Adamis *et al.*[56]. Subsequently its presence has been reported in many cells, including pericytes, endothelial cells, glial cells, Muller cells and ganglion cells within the eye[57,58]. Since it is responsive to oxygen and glucose, it is termed as oxygen and glucose responsive growth factor. Hypoxia increases adenosine levels through reduced activity of adenosine kinase. Adenosine binds through G protein coupling, resulting in increased intracellular cAMP. Activation of protein kinase C (PKC) then results in increased VEGF expression through hitherto unidentified mechanisms that upregulate VEGF elaboration in a variety of cell systems studied[59,60]. Glucose reverses this phenomenon[59]. VEGF is implicated in ischemic retinopathies such as PDR, retinopathy of prematurity (ROP) and central retinal vein occlusion (CRVO). Clinical studies on the correlation between proliferative retinopathies and intraocular VEGF concentrations have been reported[61,62]. Thus it appears that VEGF has a substantial role in the intraocular neovascularization associated with ischemic retinopathies. A comprehensive view of events leading to the development of DR is depicted in Figure 3. If these observations are true, therapeutic intervention of VEGF will benefit DM patients.

Role of oxidative stress

Oxidative stress characterized by decreased antioxidant enzymes and vitamins, has been implicated in DM, Alzheimer's disease, autoimmune diseases, infections and other diseases[63]. Metal ions, particularly Fe^{+} and Cu^{+}, participate in a process called glycoxidation, in which the carbohydrate moieties of protein are oxidized by oxygen free radicals, thus causing protein damage[64,65]. Fe^{2+} and Cu^{+} catalyse this reaction. Besides, binding of AGE to RAGE induces the free radical generation and oxidative stress to the target cells where it binds[36]. This process further leads to the *de novo* synthesis of AGE[66] (Figure 3). It has always been puzzling that although chronic hyperglycemia and increased glycation of proteins are correlated with the development of diabetic complications, there are patients in poor glycemic control who appear resistant to complications and others in good glycemic control who develop severe complications. The answer would lie in the relationship between glycation and oxidation and differences in oxidative stress among diabetic patients. If this hypothesis is correct then it supports the therapeutic approach via antioxidants for the treatment of DR.

Genetics and DR

The role of genes in the etiology of any disease is considered very important. Unfortunately, the association of potential candidate genes like insulin gene (*11p15*), maturity onset diabetes in young (MODY) genes (*20q*, *7p*, *12q*), ATP-sensitive K^{+} channel (*21q22*), glucagon-like peptide-1 (GLP-1) receptor (*6p21*), GLUT2 (*3q36*), glucose transporter (GLUT4) (*17p13*) and insulin receptor (*19p13*) with NIDDM has been ruled out (reviewed in ref. 67). Further probing into many more candidate genes by using novel methods such as polymerase chain reaction differential display, subtractive cloning and use of systemic genome scanning need to be done before concluding the association of diabetogenes with DR. These will probably require well-characterized families and patients where the relative contribution of insulin secretion and insulin resistance is better understood. Our group working on promoter regions of tumour necrosis factor (TNFα) and nitric oxide synthase (NOS) genes using microsatellite markers found that a certain allelic polymorphism predisposes/protects NIDDM subjects from developing DR (unpublished data). Polymorphism in other candidate genes like *RAGE*, *pp63* (α heat shock glycoprotein), *Rad* genes, etc. and mutational screening of these genes would also provide useful information regarding association of genetics with DR[67].

Therapeutic possibilities for DR

Surgical management of DR

Laser photocoagulation is highly effective in halting progression and often induces the regression of PDR.

Appropriately timed vitrectomy, when indicated adds a further surgical intervention in the struggle to preserve the sight in DR and include epiretinal membrane delamination, membrane peeling surgery, retinectomy, scleral buckling and endopanretinal photocoagulation. However these procedures have drawbacks resulting in post-operative haemorrhage, post-operative cataract, haemolytic glaucoma, fibrovascular proliferation, neovascular glaucoma, rhegmatogenous retinal detachment and glial recurrence[68]. Hence a new compressive therapeutic package, which can overcome the shortcomings of conventional therapeutic procedures must be developed.

Medical treatment for DR

The list of drugs given in Table 2 includes oral antidiabetic drugs, inhibitors of intermediary metabolism, antiglycating agents, growth factor inhibitors, MMP inhibitors, PKC inhibitors, antioxidants, antioxidant enzyme cofactors, and antigrowth factor antibodies. There are a few adverse effects associated with these agents (currently in use/proposed to be used) in controlling DR. Oral antidiabetic drugs like glibenclamide (sulphonyl urea) and metformin (biguanide) have been widely used in management of hyperglycemia. However sulphonyl urea produces adverse effects like diureism and weight loss. Biguanides induce vomiting, weight loss and lactic acidosis, and rarely cardiovascular mortality[69]. Ascarbose (α glycosidase inhibitor) has been found to elicit malabsorption syndrome[70]. It was thought that with the advent of insulin therapy, one could control hyperglycemia and possibly prevent development of DR in patients with type 2 DM. But further studies in this subject, led to the idea being dropped, because chronic insulin treatment itself had been attributed to the development of insulin-induced arteriosclerosis.

The use of sorbinil and other aldose reductase inhibitors has become obsolete now, because experimental evidence has shown this enzyme to be nonfunctional at levels of physiological concentration of glucose in diabetes. Aminoguanidine, an antiglycating agent, has been found to be effective in experimentally-induced diabetic nephropathy. However aminoguanidine in human clinical trials revealed that it leads to adverse hepatocellular and pancreatic toxicity[71]. Other antiglycating molecules like aspirin and ibubrufen are being widely used as cardioprotective agents and are not found to be useful for controlling hypertension[72]. But mega doses of these drugs have been found to produce gastric ulcers[73]. Supplementation of antioxidant vitamins E, C and β carotene has been found to be beneficial in patients with DM[74]. They scavenge free radical and retard the glycoxidation process. Inhibitors of MMP have been found to be antiangiogenic. The problem with MMP action is that it acts in a multifaceted manner. No single MMP has been attributed to the development of DR. So, inhibiting one particular member of MMP might not serve the purpose of therapeutic intervention of DR. Angiotensin converting enzyme (ACE) inhibitor, an anti hypertensive drug used to treat patients with type 2 DM for controlling hypertension, has been found to interfere in normal renin–angiotensin system and alter the electrolyte balance. So during the treatment of hypertension, the dosage and physiological parameters should be monitored to avoid complications.

VEGF chimeric protein, which blocks the VEGF binding to its receptor, is still in an experimental stage. Repeated injections of VEGF antibody may lead to antibody resistance. Increased rate of endophthalmitis due to repeated intraocular delivery of VEGF antibody has been reported in animal models[75]. Possibility of preventing VEGF expression by antisense VEGF mRNA is still at developmental stage[75]. Moreover, there is also neovascularization in non-ischemic regions in the retina, suggesting the involvement of other growth factors in the development of DR. The extent of the role of these growth factors would need further investigation. However growth factor antagonists have been found to impart cytotoxicity to normal retinal cells[75].

Will amino acids be beneficial in treatment/control of DR?

Free amino acids have been found to have antiglycating property[76–79]. They compete with protein amino-groups for glucose. They bind with glucose, thereby protecting tissue/cellular proteins from getting glycated[76–79]. In our experiments with animals induced with diabetes, amino acid supplementation to these animals proved to be both anticataractogenic and antidiabetic[76–79]. Further, amino acids improved the insulin sensitivity by promoting insulin receptor tyrosine kinase activity[80]. Cytoskeletal protein actin was found to be functionally impaired in granulocytes isolated from patients with diabetes. This impairment was corrected by lysine in *in vitro* studies (unpublished data). Glutathione is an important endogenous antioxidant. It is synthesized from glutamic acid, cysteine and glycine. These amino acids when supplemented orally, may improve the antioxidant status in patients with diabetes and help them to prevent/protect against lipid/protein oxidation. It is known that tissue protein synthesis is affected and metabolism becomes sluggish in diabetes. It is also true that little energy available is used for the synthesis of unwanted proteins such as growth factors adhesion molecules, proteases, etc. Our preliminary studies have indicated that amino acids augment membrane-protein synthesis when lysine was supplemented

orally to patients with type 2 DM[80]. Besides, amino acids are nontoxic, ubiquitous and non-pharmacological agents. Hence amino acids may have bright prospects in the treatment of DR. Further clinical trials with more insight towards the molecular mechanism would help in understanding the modalities of the amino acid treatment in controlling/preventing complications in DM.

Conclusions

The molecular events leading to the development of DR are indeed multifarious. These complex cascades interact in a synergistic manner towards causing DR. Though molecular mechanisms appear to be clear, the therapeutic intervention of one cascade may have an adverse influence on another. A single molecule to handle all these pathways effectively towards the treatment of DR is yet to be discovered. Glycation and glycoxidation appear to be centrally the common events in the multicascade process. Mitigation of these two events would go a long way in the treatment of DR. Among the known antiglycating molecules, consumption of amino acids is safe, as they are physiological and water-soluble. Along with amino acids, antioxidants in the form of vitamins or co-factors like zinc, in particular, can be added while monitoring their levels in circulation. Besides this, fibre-rich diet would help diabetic individuals in two ways – by improving glucose tolerance and by inhibiting glycoxidation through the removal of metals such as iron and copper. Mild exercise and regular ophthalmic check up would also be important in protecting their eyes.

Oral intake of amino acids along with routine antidiabetic medication under medical care would benefit patients with type 2 diabetes.

1. Himsworth, H. P., *Lancet*, 1936, **2**, 935–936.
2. Klein, R. and Moss, S. E., *Ophthalmology*, 1984, **91**, 1–9.
3. Klein, R. and Moss, S. E., *Arch. Ophthalmol.,* 1984, **102**, 520–528.
4. Patz, A., *Invest. Ophthalmol. Vis. Sci.*, 1980, **19**, 1133–1138.
5. Winterniz, W. W. *et al.*, *N. Engl. J. Med.*, 1976, **295**, 59–63.
6. Hotton, W. L., Snyder, W. B., Vasier, A. and Siperstein, M. D., *Trans. Am. Acad. Ophthalmol. Ortolaryngeol.*, 1972, **76**, 968–980.
7. DCCT group, *Diabetes*, 1996, **45**, 1289–1298.
8. Klein, R., Klein, B. E. K. and Demets, D. L., *J. Am. Med. Assoc.*, 1988, **268**, 2864–2871.
9. Engerman, R., Bloodworth, J. M. B. and Nelson, S., *Diabetes*, 1977, **26**, 760–764.
10. Engerman, R. and Kern, T. S., *Diabetes*, 1987, **36**, 808–812.
11. Linner, E., Svanberg, A. and Zealander, T., *Am. J. Med.*, 1965, **39**, 298–304.
12. Kroleski, A. S. *et al.*, *Diabetes Care*, 1986, **9**, 443–452.
13. Dahl, J. K. and Brinchmann, T., *Br. Med. J.*, 1985, **290**, 811–815
14. Heyningen, V. R., *Nature,* 1959, **184**, 194–196.
15. Sullis, L. P., Cooper, T. M. E., Dunlop, M. J. and Jernus, G., *Diabetalogia*, 1995, **38**, 387–394.
16. Brownlee, M., Cerami, A. and Vlassara, H., *N. Engl. J. Med.*, 1988, **318**, 1315–1321.
17. Horichi, S. *et al.*, *J. Biol. Chem.*, 1988, **263**, 1821–1826.
18. Ramanakoppa, H. N., *J. Biol. Chem.*, 1998, **273**, 6928–6936.
19. Vlassara, H., *Diabetes*, 1997, **46**, S19–S25.
20. Vlassara, H., Brownlee, R. and Cerami, A., *J. Exp. Med.*, 1986, **164**, 1301–1309.
21. Sulochana, K. N., Nagaraj, R. H. and Mruthinti, S., *Invest Ophthalmol. Vis. Sci. (Abstr.),* 2000, **41**, 4807.
22. Vlassara, H., *Blood Purif.*, 1994, **12**, 54–59.
23. Bucla, R., Mukita, Z., Vega, G., Grundy, S., Koschinsky, Cerami, A. and Vlasara, H., *Proc. Natl. Acad. Sci. USA*, 1994, **91**, 9441–9445.
24. Alan, W. S. and Moore, J. E., *Invest Ophthalmol. Vis. Sci.*, 1998, **39**, 2523–2571.
25. Hammes, H. P. *et al.*, *Diabetologia*, 1999, **42**, 728–736.
26. Yamagishi, S., Kobayashi, K. and Yamamoto, H., *Biochem. Biophys. Res. Commun.*, 1993, **190**, 418–425.
27. Rasmussen, F., *Diabetalogia*, 1986, **29**, 282–286.
28. Kuwabara, T. and Kogan, D. G., *Arch. Ophthalmol.*, 1960, **64**, 904–911.
29. Antoneliorlidge, A., Saunders, K. B., Smith, S. R. and D'Amore, P. A., *Proc. Natl. Acad. Sci. USA*, 1989, **86**, 4544–4548.
30. Orlide, A. and D'Amore, P. A., *J. Cell Biol.*, 1987, **105**, 1455–1462.
31. Wallow, I. H. and Brownside, B., *Invest Ophthalmol. Vis. Sci.*, 1980, **19**, 1433–1441.
32. Schmidt, A. M. *et al.*, *J. Biol. Chem.*, 1992, **267**, 14897–14997.
33. Yamagishi, S. *et al.*, *Biochem. Biophys. Res. Commun.*, 1995, **213**, 681–689.
34. Yamagishi, S. *et al.*, *J. Biol. Chem.*, 1997, **272**, 8723–8730.
35. James, T. H., Karen, M., Hiroshi, M. and Leonard, M., *Exp. Eye Res.*, 1998, **64**, 411–419.
36. Schmidt, A. M. *et al.*, *Proc. Natl. Acad. Sci. USA*, 1994, **91**, 7742–7746.
37. Woessner, J. F., *FASEB J.*, 1991, **5**, 2145–2154.
38. Nagase, H., *Matrix Metalloproteases in Health and Diseases* (ed. Hooper Nill), Taylerad, UK, 1996.
39. Stevenson, S. W. G., Liotta, L. A. and Lecher, D. E. K., *FASEB J.*, 1993, **7**, 1434–1441.
40. Arup Das, *et al.*, *Invest Ophthalmol. Vis. Sci.*, 1998, **40**, 810–813.
41. Liotta, L. A., Steeg, P. A. and Stevensen, S. W. G., *Cell*, 1991, **64**, 327–336.
42. Rosen, E. *et al.*, *Ciba Found. Symp.*, 1997, **212**, 215–222.
43. Hansen, H. B. *et al.*, *Crit. Rev. Oral Biol. Med.*, 1993, **4**, 197–250.
44. Lynn, A., *J. Immunol.*, 1998, **101**, 6845–6852.
45. Burn, K. M., *Curr. Opin. Cell Biol.*, 1990, **2**, 857–863.
46. Mantesano, R., Vassuli, J. D., Baid, A., Guillemin, R. and Orchi, L., *Proc. Natl. Acad. Sci. USA*, 1986, **83**, 7297–7301.
47. Baird, A. and Walicke, P. A., *Br. Med. Bull.*, 1989, **45**, 438–452.
48. Abraham, J. A. *et al.*, *Science*, 1986, **233**, 545–548.
49. Sivalingam, A., Kenny, J., Brun, G. C., Bensen, W. E. and Donoso, L., *Arch. Ophthalmol.*, 1990, **108**, 864–872.
50. Grant, M., Russell, B., Fitgerald, C., Ellis, E. A., Cubullerous, A. and Guy, J., *Ann. N.Y. Acad. Sci. USA*, 1993, **962**, 230–242.
51. Grant, M., Russell, B. and Fitgerald, C., *Diabetes*, 1986, **35**, 416–420.
52. Dills, D. G., Moss, S. E. and Klein, R., *Diabetes*, 1991, **40**, 1725–1730.
53. Wong, O., Dills, D. G., Klein, R. and Moss, W., *Diabetes*, 1995, **44**, 161–164.
54. Robins, S. G. *et al.*, *Invest Ophthalmol. Vis. Sci.*, 1994, **35**, 3640–3663.

55. He, P. *et al.*, *Biochem. Biophys. Res. Commun.*, 1998, **249**, 253–257.
56. Adamis, A. P. *et al.*, *Biochem. Biophys. Res. Commun.*, 1993, **193**, 631–638.
57. Simorre, P. *et al.*, *Invest Ophthalmol. Vis. Sci.*, 1947, **35**, 36–47.
58. Lotty, G. A. *et al.*, *Arch. Ophthalmol.,* 1996, **114**, 971–977.
59. Stein, I., Niceman, M., Shweiki, D. and Kesht, E., *Mol. Cell Biol.*, 1995, **15**, 5363–5368.
60. Goldberg, M. A. and Schneider, T. J., *J. Biol. Chem.*, 1994, **296**, 4355–4359.
61. Adamis, A. P. *et al.*, *Am. J. Ophthalmol.*, 1994, **118**, 445–450.
62. Aiello, L. P. *et al.*, *N. Engl. J. Med.*, 1994, **331**, 1480–1487.
63. Hailliwel, B., Gutteridge, J. M. C. and Cross, C. E., *J. Lab. Clin. Med.*, 1992, **119**, 598–620.
64. Wolf, S. P. and Dean, R. T., *Biochem. J.*, 1987, **254**, 243–248.
65. Wellsknect, M. C., Thrope, S. R. and Baynes, J. W., *Biochemisty,* 1995, **64**, 411–419.
66. Lyons, T. J. and Jenkins, A. J., *Diabetes Rev.*, 1997, **5**, 365–391.
67. Kahn, C. R., Vincent, D. and Doria, A., *Annu. Rev. Med.*, 1996, **47**, 509–531.
68. Steve, T., in *Diabetic Renal Retinal Syndrome* (eds Friendman, Eli A., Frances, A. L. and Penance, J.), Kluwer Academic Press, UK, 1998, 1st edn.
69. Reynolds, J. E. F., *Martindale Pharmacopoeia*, The Pharmaceuticals Press, UK, 1990, pp. 386–399,
70. Baron, A. and Neuman, C., *Clin. Ther.*, 1997, **19**, 282–295.
71. Sandler, H. M. S., *Autoimmunity*, 1993, **15**, 311–314.
72. ETDRS investigators, *J. Am. Med. Assoc.,* 1992, **268**, 1292–1300.
73. Reynolds, J. E. F., *Martindale Pharmacopoeia*, The Pharmaceuticals Press, UK, 1990, pp. 20–21.
74. Taylor, A., *Ann. N.Y. Acad. Sci. USA*, 1991, **669**, 111–124.
75. Aiello, L. P., *Curr. Opin. Ophthalmol.*, 1997, **8**, 19–31.
76. Ramakrishnan, S. and Sulochana, K. N., *Exp. Eye Res.*, 1993, **57**, 623–628.
77. Ramakrishnan, S., Sulochana, K. N., Punitham, R. and Arunagiri, K., *Glycoconjugate J.*, 1996, **13**, 519–523.
78. Ramakrishnan, S., Sulochana, K. N. and Punitham, R., *Indian J. Biochem. Biophys.*, 1997, **34**, 518–523.
79. Sulochana, K. N., Punitham, R. and Ramakrishnan, S., *Exp. Eye Res.*, 1998, **67**, 597–601.
80. Rajesh, M., Sulochana, K. N. and Ramakrishnan, S., Abstract, 68th Annual Meeting of SBC(I), Bangalore, 1999, D8.
81. Zeffer, B. R., *Chest*, 1998, **93**, S15–S66.
82. Busilico, C. and Mascatelli, D., *Adv. Cancer Res.*, 1992, **59**, 115–165.
83. Raines, E. W. and Bowenpope, D. F., *Cell*, 1986, **44**, 155–159.
84. Riasu, W. *et al.*, *Growth Factors*, 1992, **7**, 261–266.
85. Roberts, A. B. *et al.*, *Proc. Natl. Acad. Sci. USA*, 1986, **83**, 4167–4171.
86. Fajardo, L. F. and Grave, G. E., *Am. J. Pathol.*, 1992, **140**, 539–544.
87. Ferrara, N., *Endocr. Rev.*, 1992, **13**, 18–32.
88. Nako, H. J., Ito, H., Kanayasu, T. and Marota, S., *Atherosclerosis*, 1992, **92**, 141–149.
89. Kook, Y. H. *et al.*, *EMBO J.*, 1994, **13**, 3983–3991.
90. Hynes, R. O., *Cell*, 1992, **69**, 11–26.
91. Daniel, T. P. and Loscalzo, J., *Am. J. Med.*, 1999, **107**, 85–97.
92. Relly, M. S. *et al.*, *Cell*, 1994, **79**, 315–328.
93. Relly, M. S. *et al.*, *Biochem. Biophys. Res. Commun.*, 1995, **217**, 326–332.
94. Roberts, D. D., *FASEB J.*, 1996, **10**, 1183–1191.
95. Fukuski, S., Merola and Kinoshita, *Invest Ophthalmol. Vis. Sci.*, 1980, **19**, 313–315.
96. Raved, M., Leng, R., Rachmani, R. and Leischmer, M., *Arch. Intern. Med.*, 1996, **156**, 286–289.
97. Thompson, D. W., William, W. and Maragoudakis, M., *J. Pathol.*, 1999, **25**, 503–510.
98. Vlassara, H., Brownlee, M. and Manouge, K., *Science,* 1988, **240**, 1540–1548.
99. Beachy, N. A. *et al.*, *Photochem. Photobiol.*, 1987, **45**, 677–678.
100. Aiello, L. P. *et al.*, *Proc. Natl. Acad. Sci. USA*, 1995, **92**, 10457–10461.
101. Adamis, A. *et al.*, *Arch. Ophthalmol.*, 1996, **114**, 66–71.
102. Robinson, G. S. *et al.*, *Proc. Natl. Acad. Sci. USA*, 1996, **93**, 4851–4856.
103. Gagliardi, H. H. and Collins, D. C., *Cancer Res.*, 1992, **52**, 5073–5076.
104. Coombe, D. R. *et al.*, *Int. J. Cancer*, 1987, **39**, 82–88.
105. Pladder, J. M., *Sem. Oncol.*, 1997, **24**, 203–218.
106. Caravan, P. A. *et al.*, *J. Diabetes Complications*, 1995, **9**, 241–245.
107. Passanti, A., *Lab. Invest.*, 1992, **67**, 519–528.
108. Baynes, J. W. and Thrope, S. R., *Diabetes*, 1999, **48**, 1–9.
109. Smith, V. A., Hoh, H. B. and Easty, D. L., *Br. J. Ophthalmol.*, 1999, **83**, 1376–1383.

Received 16 October 2000; revised accepted 6 December 2000