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Review Article

Pathophysiology and Management of Diabetic Retinopathy Features

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Abstract

The Diabetic Macular Edema (DME) is an ischemic degenerative inflammatory pathology that leads to edematous vascular leakage in the layers of the neuro-retina, which is associated with degeneration of photoreceptors. As a result, the DME is the leading cause of vision loss in people with diabetes. The alteration of the retinal vascular barrier is the hallmark of this disease characterized by a loss of pericytes with rupture of endothelial cell junctions. Multiple cytokines and chemokines are involved in the pathogenesis of this disease with multiple cellular implications affecting the neurovascular unit. Angiography, optical coherence tomography (OCT) and genetic immunohistochemistry tests (to observe the rate of apoptosis of nervous and vascular cells) are the most relevant diagnostic tests. To date, given the multifactorial nature of diabetes and even with the revolutionary introduction of Anti-VEGF, there is no treatment robust enough to remedy the exacerbation of the fluid. New potential therapies targeting molecules other than VEGF and based on automatic dose delivery systems are currently being tested.

This review herein expands on the pathophysiological mechanisms underlying the DME, as well as the new therapeutic approaches currently under development or to be developed in order to deduce, among a great variability, the most effective indication for each patient individually, taking into account his genetic and immune profile.

Keywords: *Diabetic Macular Edema; Retinal Vascular Barrier; Permeability; Inflammation; Apoptosis; Homeostasis*

Epidemiology

Diabetes is the global epidemy of the 21st century. At present, there are 382 million people with diabetes worldwide. This number may rise to 592 million in 2035 [1]. Retinopathy is one of the most common complications of diabetes, affecting ~ 20% of adults with diabetes [2]. Diabetic retinopathy (DR) is a microvascular complication characterized by chronic inflammation, retinal visceral and neuronal degeneration, vascular leakage leading to diabetic macular edema (DME) resulting in ischemia and poor blood distribution to retinal tissues compensated by neovascularization. This latter proliferative stage is the major cause of vision loss with a greater incidence and greater risk compared to other diabetic eye risks such as vision fluctuations, cataracts and glaucoma.

Among diabetic patients, the general prevalence of DR is 35%, proliferative DR (PDR) is 6.96%. The Vision is damaged in 10.2% of DR, with DME present in 25% of diabetics [3] affecting the central vision with metamorphopsia and micropsies [4]. Levels are higher in type 1 diabetics than in type 2 [3]. According to the results of the Wisconsin epidemiological study of diabetic retinopathy, the incidence of type 1 DME over 25 years was 29%.

According to the Wisconsin epidemiological study of DR (WESDR) over a large population, the incidence of retinopathy is rarely detected in the early years of diabetes, but 80% of type 1 diabetes develops retinopathy in the first 10 years, of which 27% have DMEs. The incidence amounts to 95% of DR in 25 years, of which 29% have DME. 50% of these cases progress to a PDR after 15 years of diabetes.

Type 2 diabetes is more frequent than the type 1, it accounts for 90% of the cases. 60% of type 2 diabetic patients develop DR, which is the most common microvascular complication in these patients, of which 25.4% of DME (if insulin is needed) or 13.9% (if insulin is not needed). 10% of the DMEs evolve to a PDR.

The DME touches: 3% of the mild Non-Proliferative DRs (NPDR), 38% of moderate to severe NPDR, 71% of PDR. The existence of the DME is strongly correlated with the duration of diabetes and glycemic control. Its prevalence increases from 5% of individuals at 5 years after diagnosis to 15% at 15 years.

The DME can intervene in the three stages of DR: NPDR, preproliferative DR and/or PDR (Figure 1). Its description is better elaborated in the NPDR.

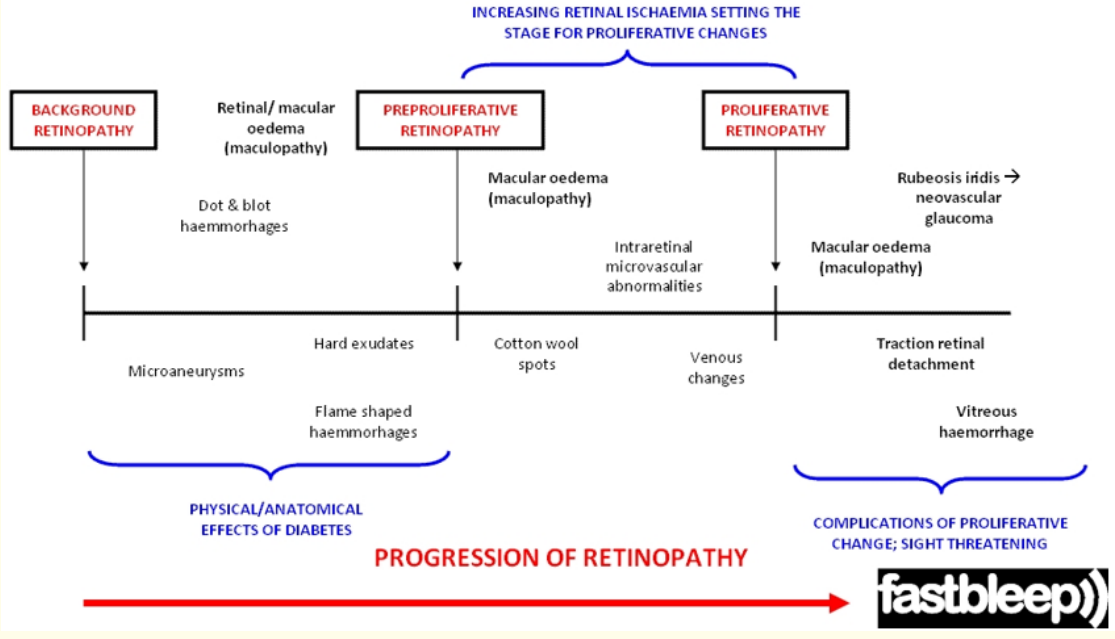


Figure 1: *Progression of the retinopathy.*

Clinical classification of the DME

The DME can be classified according to the distance of the thickening and/or the affectation of the center of the macula (fovea). It is said "weak" in case of retinal thickening or hard exudates (lipids) in the posterior pole but distant from the fovea. It is said "moderate" near the fovea and "severe" if the fovea is affected. In the latter case the patient becomes symptomatic with metamorphopsia and loss of vision.

The DME is classified as "focal" or "diffuse" according to the source of fluorescein disruption (reflecting the degree of vascular permeability therefore the degree of rupture of the retinal vascular barrier: In the ETDRS, it is "focal" when more than 67% of rupture are associated with microaneurysms (identifiable source of rupture), "intermediate" for 33 - 66% and "diffuse" for those < 33% (sources of multiple and unidentifiable fractures) [3]. The focal DME is associated with better visual acuity, minor macular thickening and a less severe DR Often the DME has characteristics of the 2 forms thus making the distinction difficult. There is no difference regarding the therapy. It is called "cystoid" when the fluid accumulates in the outer plexiform layer and the inner nuclear layer to form cystoid spaces.

The ETDRS (Early Treatment Diabetic Retinopathy Study) defines a "clinically significant" DME in 3 cases:

- The thickening of the retina occurs in the 500 um of the fovea.
- The lipidic deposits occur within 500 µm of fovea distance if associated with adjacent retinal thickening.

- If there is an area of retinal thickening of 1disc surface, part of which is located in the 1 diameter disc of the fovea.
- The examination of the DME requires stereoscopic biomicroscopy of the macula or a stereoscopic photograph of the fundus.
- The existence of the DME is strongly correlated with the duration of diabetes and blood glucose control.

Factors of susceptibility and severity

A study of Kaidonis G., *et al.* 2014 showed that the presence of diabetic retinopathy that threatens vision (severe non-proliferative, proliferative, DME) is significantly correlated with the duration of diabetes mellitus, hypertension, nephropathy, HbA1C and the BMI. The DME was associated with type 2 diabetes ($P < 0.001$), whereas PDR was associated with type 1 diabetes ($P < 0.001$) [5] DR almost always occurs in type 1 diabetes and in 80% of type 2 diabetes.

- The elevated concentration of glycosylated hemoglobin (HbA1c), as well as the concentrations of IGF-1 and insulin, which lead to overexpression of the VEGF whose retinographic results are cotton-wool spots and hemorrhage spots.
- Metabolic memory: Strict glucose control protects against the development of DR by 76% and slows its progression by 54% [1].
- Hypertension: increases hydrostatic forces [1].
- Hypoalbuminemia, Hyperlipidemia.
- The serum or plasmic fibrinogen level secreted by Muller cells: it is associated with the risk of basement membrane thickening and epiretinal membrane formation.
- The vitreous detachment (The axial high myopia): Longer axial length predicts a better BCVA after vitrectomy of diffuse macular edema secondary to DR [35]. On the other hand, in myopic eyes, the vitreous, which contains 10 times more cytokines and toxic glycated products (AGEs) under the influence of diabetes, is detached from the retina by Posterior Vitreous Detachment (PVD). This prevents these inflammatory and apoptotic molecules from infiltrating the retina. Thus, the highly axial myopia is also a protective factor against DR.
- Genetic factors: like the SNP (Single Nucleotide Polymorphism) C-634G (genotype CC) of the VEGF or the C allele of rs2010963, they are the polymorphisms of the risk, prevalent among the DME patients ($P = 0.019$). Larger levels of VEGF are detected in their serum ($P = 0.02, 0.016$ [6]. To date, 7 forms of VEGFα splicing have been identified, including the deleterious VEGF165 of exon 6, VEGFAβ, VEGFA165b. More than one SNP of VEGFα is independently associated with the risk of developing severe RD in type 1 diabetes [7].

The A-2518G polymorphism of the CCL2 gene, a chemoattractant of macrophages, is directly associated with increased expression of CCL2 and recognized as a potential risk factor for DR. Polymorphism may explain in part the highest prevalence of DR in the overseas departments of France (DOM-TOM). Also, the polymorphism of the erythropoietin gene promoter is correlated with the evolution towards a PDR. This risk is low among Mexicans, Americans, Chinese and the White population for a PDR but no correlation for the incidence of DME; The waist/hip ratio is also a predisposing factor because it determines the amount of fat tissue as a result of the level of inflammation; There is more adipose tissue in women, so women are more at risk than men.

Molecular variations in diabetes

Visualization on electrophoresis gel allowed identifying and measuring the levels of variations of plasma and vitreous molecules. Four proteins are specifically associated with DME [8]: Hemopexin significantly higher in vitreous fluid, it could mediate rupture of the retinal vascular barrier and it appears to be directly related to the development of DME [9,10]. Transthyretins and β-crystallin S are significantly reduced in vitreous DME patients [8].

In addition, eight other proteins are amplified in DME samples. Among these proteins, six have been identified: PEDF (Pigment Epithelium Derived Factor), ApoA-4, ApoA-1 (Apolipoproteins), Trip-11, PRBP, and VDBP. In contrast, Apo H is completely repressed in DME patients. These posterior vitreous mediators play a role in the pathogenesis of DME [11].

Chemokine levels are also increased in the vitreous fluid, VEGF (Vascular Endothelial Growth Factor), ICAM-1 (intercellular adhesion molecule-1, mediator of adhesion of leucocytes to the endothelium, resulting in damage to the vasculature, non-perfusion of the capillaries and hyperpermeability), Ang 2 (Angiotensin-2), IL-6 (interleukin 6), and MCP-1 also named CCL2 (monocyte/macrophage

chemoattractant protein-1, primary responsible for the influx of leukocytes at sites of inflammation in tissues) especially with the CCL2 gene polymorphism A-2518G [12]. The Significantly high VEGF levels (3 to 30 times higher) were correlated with the severity of vascular leakage and hyperpermeability in diabetic eyes with DME: VEGFA is a mitogen that specifically acts on ECs and mediates vascular hyperpermeability, induces angiogenesis, cell development, migration and inhibition of apoptosis. In retinal cells the expression of VEGFA is increased by 3 to 30 fold by hypoxia and DR, especially in PE, glial cells and vitreous fibroblasts. The yet higher plasma and vitreous levels of VEGFA, corresponded to PDR [7].

Highly correlated molecules with retinal thickness at the foveal center, in relation to vascular permeability and the severity of DME: they are the TNF-α (Tumor Necrosis Factor-alpha), IL-1β (interleukin-1β), significantly more expressed in DME. However, PEDF levels are significantly reduced in the DME, proportionally to hyperfluorescence (severity).

VEGF and ICAM-1 have a greater influence on the severity of the EMR than other factors [13].

Biochemistry and pathogenesis of DME

Several signaling pathways activated by hyperglycemia cause microvascular damage in the retina, inflammation, atrophy of the PE and neuro-retina, rupture of the blood-retinal barrier, and as a result, hyperpermeability (three alterations: loss of cellular junctions, loss of pericytes and fibrotic thickening of the basement membrane) and accumulation of fluids in the extracellular space that can lead to cystoid changes with a vitreomacular interface especially in the chronic form of DME.

In the most severe cases (greater activation and secretion of effectors over a longer period), further complications are added: vitreous hemorrhage following neovascularization to the vitreous. These new blood vessels tend to be more permeable and therefore more vulnerable to rupture; tractional retinal detachment when edema separates the PE from the retina.

Polyols and PKC-VEGF pathways

Under normal conditions, glucose takes the oxidation pathway by hexokinases, only very few go through the aldose reductase (AR) pathway. Under conditions of hyperglycemia, leading to recurrence of diabetes, the hexokinase pathway is saturated, forcing glucose to enter the AR pathway (Figure 3) [14,15]. This already reduces the ability of cells to respond to oxidative stress due to the greater conversion of NADPH to NADP+. Sorbitol produced from glucose by the AR is then metabolized to fructose resulting in a rise in the NADH/NAD + level, a condition called “pseudohypoxia” [14], resulting in the generation of intracellular oxidative species of ROS (reactive oxygen species), of sorbitol, precursors of AGEs (Advanced Glycated Ends, toxic: break the function of the proteins and interfere with the membrane receptors) and diacylglycerol (DAG) by making available their substrates, and block the cycle at this level. Indeed, sorbitol is a polyhydroxy agent and highly hydrophilic alcohol that does not diffuse immediately through the cell membranes, therefore accumulates in the cytosol and induces osmotic stress, making the membrane more permeable leading to cell damage (osmotic theory of polyols) (Figure 4) [14,15]. Its excessive accumulation induces irreversible damage, ending in pericyte loss, thickening of the basement membrane and DME.

NB: AGEs that normally accumulate in the extracellular matrix with age, accumulate very early in the extracellular matrix of the diabetic, become encrusted, establish irreversible cross-links with collagen in the long term and reduce the susceptibility of the membrane to proteolysis. They are correlated with the severity of retinopathy. AGEs decrease the electronegative charges of the vascular membrane and alter its permeability (filtration properties) thus leading to a DME. Capillary closure leads to non-perfused spaces (ischemic retinopathy) stimulating VEGF secretion and neovascularization reaching the PDR stage [14].

AGEs are also formed on the amino groups of lipids and DNA, resulting in alterations in the function, activity and degradation of intra- and extracellular proteins via chemical rearrangements and intra-or intermolecular cross-linking with collagen, elastin from the retinal vascular barrier, thereby altering its structural integrity.

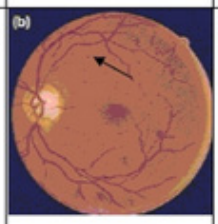
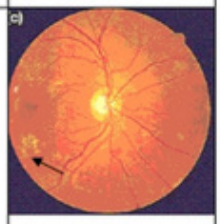
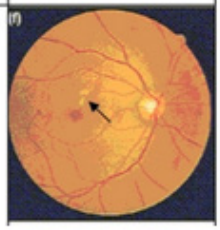
Grade of retinopathy	Features	Appearance	Fundoscopy	Pathophysiology
Non-proliferative	Microaneurysms	Small red dots in superficial retinal layers.		Outpouching of the capillary wall due to pericyte loss.
	Dot & blot haemorrhages	Appear similar to microaneurysms if small.		Microaneurysm rupture in the deeper retinal layers.
	Flame-shaped haemorrhages	Splinter haemmorhages		Microaneurysm rupture in superficial nerve fibre layer.
	Retinal oedema	Dull appearance to the retina.		Leakage of serum proteins, lipids & protein from vessels due to breakdown of blood-retina barrier.
	Hard exudates	Waxy, yellow lesions often arranged in clumps or rings.		
	Macular oedema	Retinal thickening at macula, hard exudates within disc width of the macula.		

Figure 2: *Symptoms of the DR.*

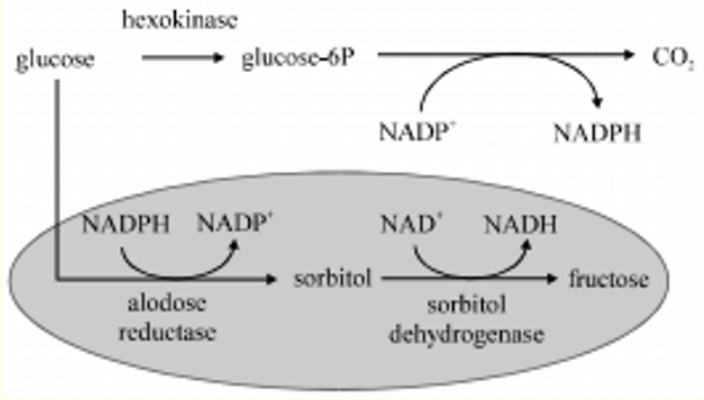


Figure 3: *Hexokinases pathway and polyol pathway for glucose oxidation. In diabetes, the Aldose reductase pathway prevails [15].*

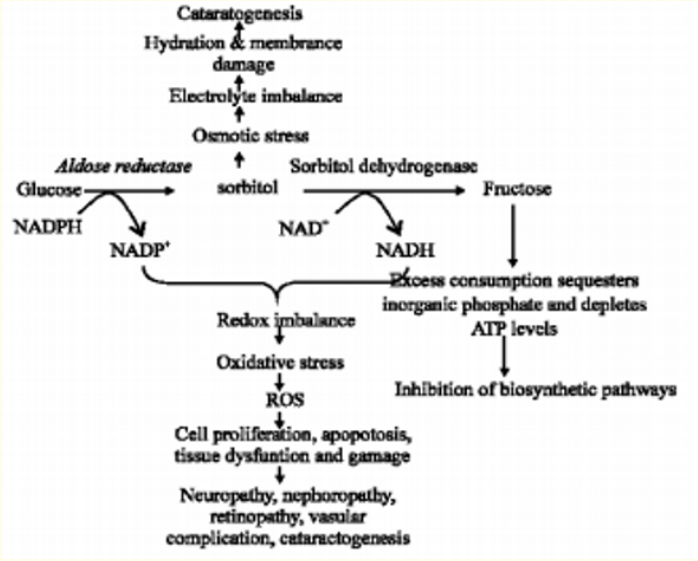


Figure 4: *Polyol pathway and its effects [15].*

AGEs and DAG activate the AGE pathway and the PKC (protein kinase C) pathway in endothelial cells (Figure 5 and 6). PKC decreases angiotensin II contractile vascular response and activates sodium-proton pumps that regulate cell pH, Cellular development, differentiation and increased expression of matrix proteins such as fibronectins and type 4 collagen which means thickening of the basement membrane and epiretinal membrane formation.

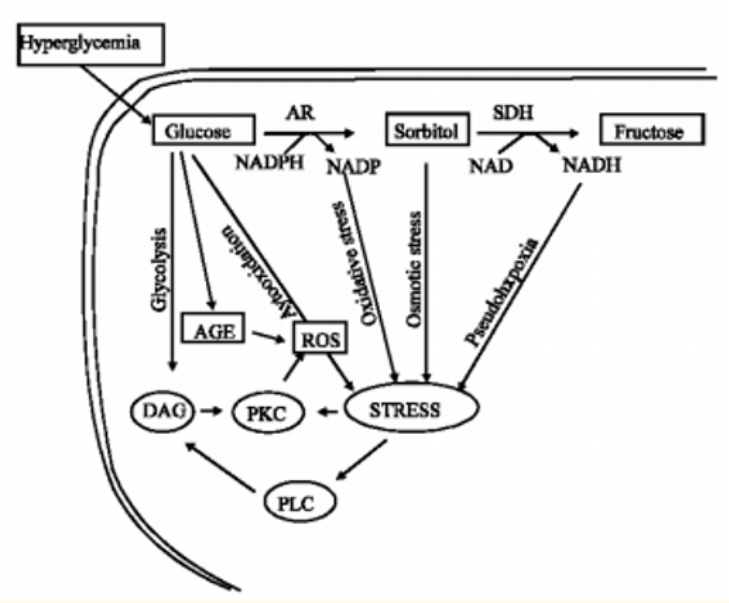


Figure 5: *Mechanisms of induction of oxidative stress by hyperglycemia [15].*

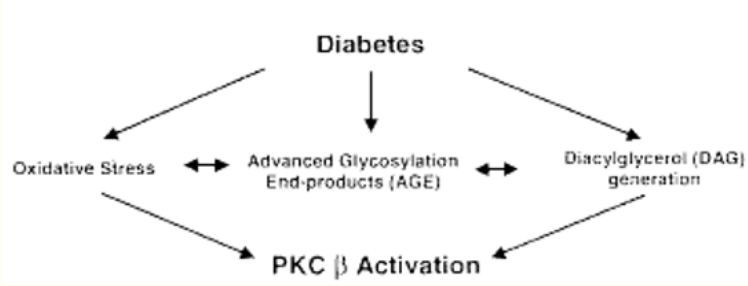


Figure 6: *Mechanisms of induction of PKC-β activation [15].*

PKC pathway activates leucocytes and increases cytokine secretion; promotes endothelial proliferation and apoptosis by regulating the action of factors such asVEGF, IGF-1 (Insulin-growth factor-1) and TGFβ1 (Transforming Growth Factor β1) (Figure 7-9). The activation level of PKC is proportional to the severity of the DME AR is expressed in the PE, retinal nerve cells and in the lens (hence the formation of cataracts), in pericytes (hence their degradation due to AR-catalyzed accumulation of AR sugars); and in erythrocytes. A therapeutic approach would be to inhibit the production of AGEs by anti-AGER antibodies (antibodies to the AGE receptor), or to produce AR inhibitors, DAG inhibitors or PKC-beta inhibitors [14].

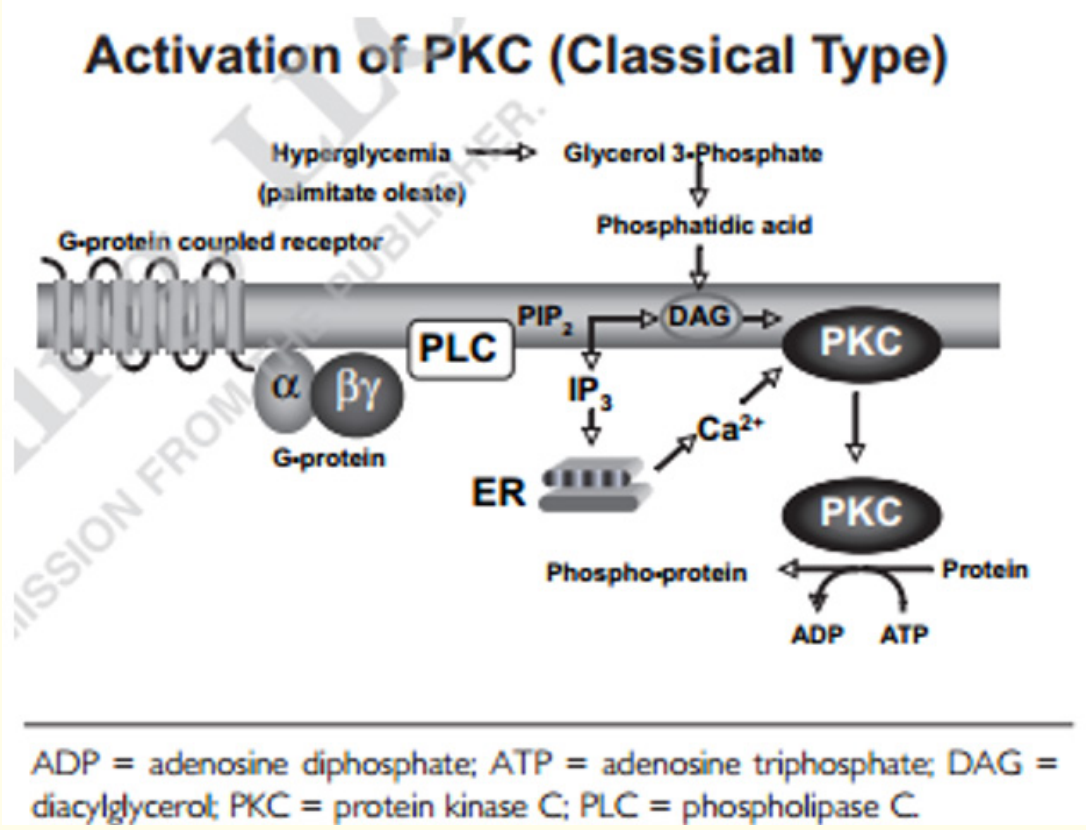


Figure 7: *Activation of PKC induced by hyperglycemia.*

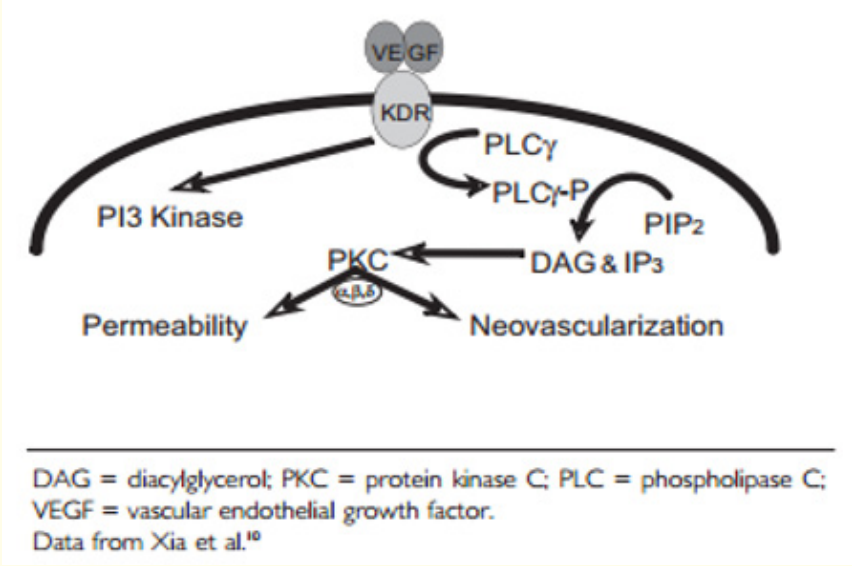


Figure 8: *Mechanism of action of VEGF on PKC.*

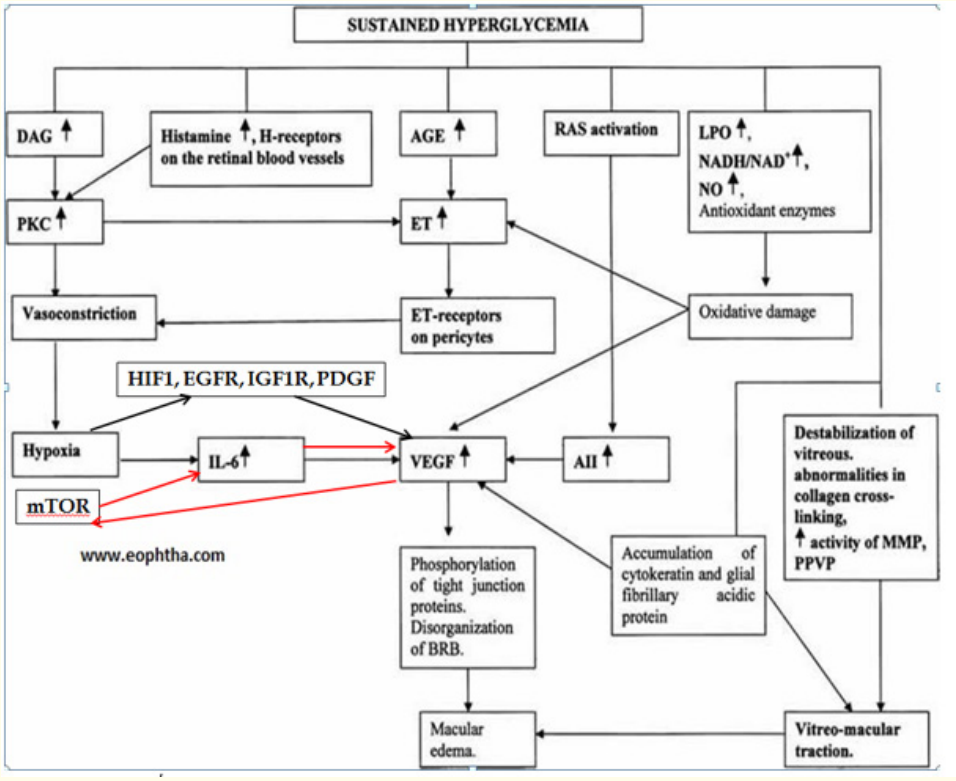


Figure 9: *Recapitulative scheme of DME induction.*

The oxidative and mitochondrial stress

Possible sources of oxidative stress in diabetes are: auto-oxidation of glucose, disruption of the mitochondrial electron transport chain, redox imbalances, reduction of tissue concentration to low molecular weight anti-oxidants normally present, responsible for the regulation of redox homeostasis, such as reduced glutathione (GSH), vitamin E, vitamin C, and β-carotene; a disruption of the function of the enzymatic antioxidants of defense such as superoxide dismutase (SOD), glutathione reductase, glutathione peroxidase and catalase which are responsible for the elimination of the free radicals, the maintenance of the homeostasis redox. In one experiment, the intracellular glutathione were depleted in the retina 2 months after induction of diabetes but not in the brain, suggesting that oxidative stress is more pronounced in the retina [1].

The consequences of chronic oxidative stress lead to a modulation of macrobiological molecules such as the DNA (mutations, repression, stimulation of expression of growth factors...), lipids, proteins and carbohydrates, rupture of cellular homeostasis and generation of other ROS.

The generation of ROS puts a stop to the activation of the VEGF-R2 signaling pathway, which increases the breakdown of the retinal endothelial cell barrier [15].

Hexoamines pathway

The inhibition of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and hyperglycemia-induced superoxide production contributes to activation of the hexoamine pathway that activates the AGE pathway. This pathway induces post-translational modifications of intracellular factors including transcription factors of cytokines, chemokines and inflammatory proteins.

Endoplasmic Reticulum stress (ER) vs response of non-folded proteins and inflammation:

The pathological conditions of diabetes, such as accumulation of AGEs, mutant proteins, energy and nutrient deprivation and alteration of the redox status, compromise the effectiveness of ER in protein folding resulting in accumulation of mis-refolded proteins in the ER lumen: ER stress (N.B: it is the repetitive fluctuations of high glucose to normal glucose that induce ER stress, not the constant high glucose levels) [12,13] (Figure 10).

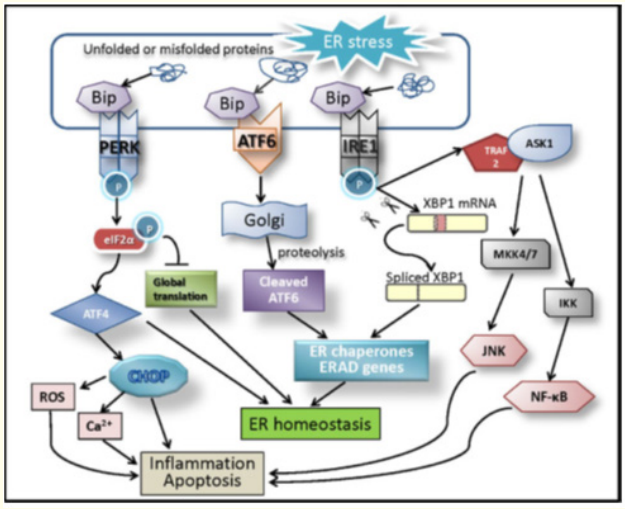


Figure 10: *UPR signaling pathway activated by ER stress in inflammation and apoptosis. The accumulation of non or folded proteins in the ER lumen sensitizes 3 ER membrane proteins: IRE1, PERK, and ATF6 [9].*

This results in an increase in the expression of ICAM-1 and VCAM-1 (adhesion molecules that call for leukocytes to adhere, breaking the tight junctions of the retina allowing blood leakage and endothelial apoptosis), ROS generation and predisposes cells to apoptosis induced by inflammation (Figure 11) [9].

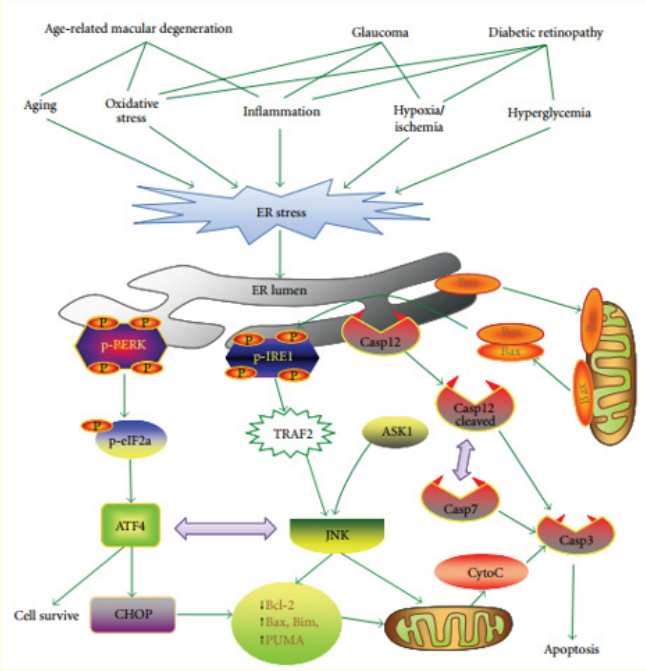


Figure 11: *apoptosis pathways associated with ER stress in retinal diseases [28].*

Zhang., *et al.* have demonstrated for the first time that several ER stress markers were significantly overexpressed in the retina. The induction of ER stress is sufficient to induce the expression of inflammatory genes in the retina such as TNF-α (x 5.1) and VEGF (x 4.3) [9]. Overexpression of TNF-α suppresses the expression of claudin-5, a major junction protein, contributing to hyperpermeability in DR.

Pericytes apoptosis and vascular leakage

The retinal vascular barrier groups the internal barrier located mainly at tight junctions between adjacent ECs; and the outer barrier formed by the cells of the PE. Pericytes are contractile cells that surround the endothelial cells of capillaries and venules. They regulate blood flow, phagocytosis of cellular debris and membranous permeability. The rupture and apoptosis of the retinal barrier in diabetes occurs very early in diabetes (in less than 1 month of hyperglycemia, even before retinopathy in diabetics). It is characterized by a loss of pericytes and a rupture of cellular junctions. These changes are among the earliest events in DR (after apoptosis of neuro-retinal cells) (Figure 12) [2].

This rupture of the junctions increases the permeability of the vessels, lets escape plasma molecules that will accumulate in the extracellular space of the retina, as a result of the swelling of the glial and neuronal cells in the macula; On the other hand, this rupture allows blood components to diffuse from the outer barrier to the neuro-retina through a DME, contributing to retinal thickening (Figure 13 and 14) [12]. Retinal vasculature devoid of pericytes showed characteristic signs [2] (Figure 14): disorganized vascular networks with dilatation and turbulence, progressive extravasations with DME and hemorrhages, thickening of perendothelial basement membranes

(Figure 15) [29]. The increase in ischemia secondary to the massive fall of retinal capillaries is a significant predictor of progression to proliferative retinopathy (PDR) characterized by neovascularization in response to vasogenic factors.

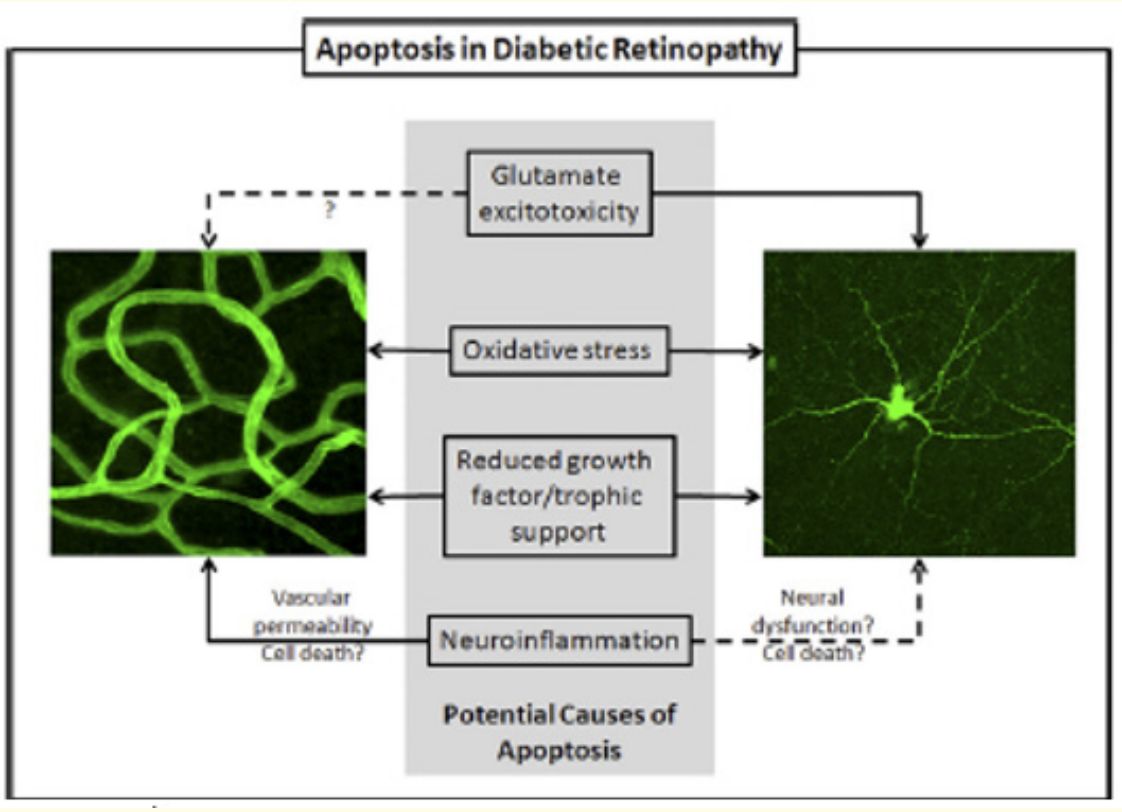


Figure 12: *Potential causes of apoptosis in DR [2].*

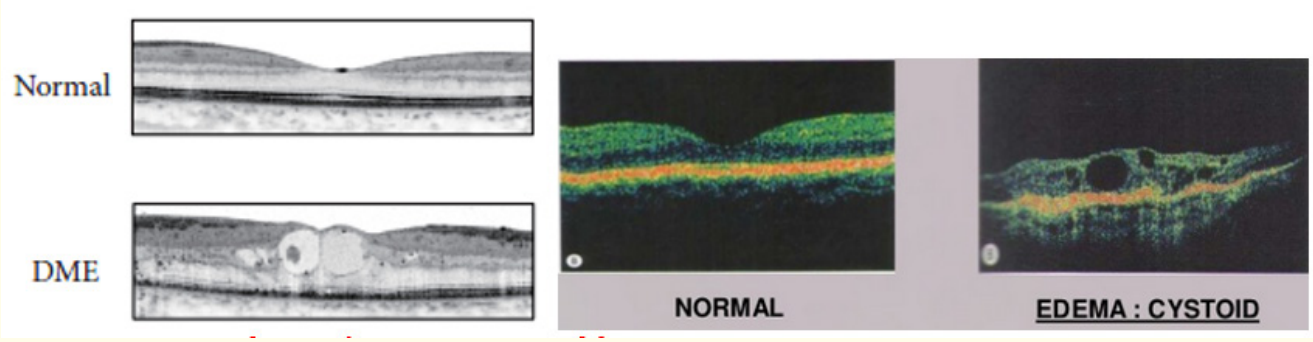


Figure 13: *OCT image of a normal retina vs DME retina [4].*

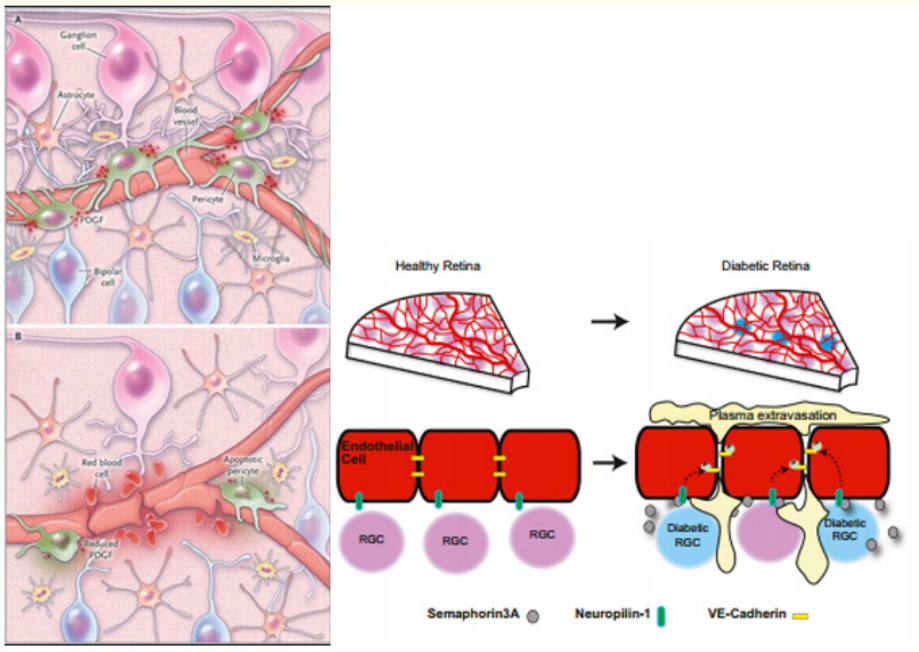


Figure 14: *Left: A modest but significant increase in vascular apoptosis suggests a potential mechanism for the appearance of acellular capillaries (as endothelial cells and nuclei disappear) in which the basement membrane or cytoplasm of residual endothelial cells remains that the nucleus is absent. Apoptosis participates in the appearance of pockets in the basement membrane. Right: plasma extravasation through the retinal barrier made permeable by modification of tight junctions in the diabetic retina [31].*

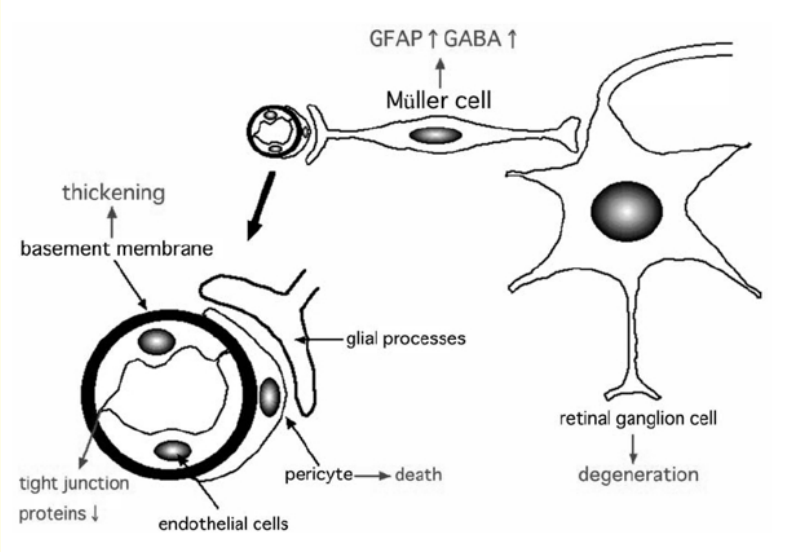


Figure 15: *Thickening of the basement membrane of the retinal vessels [29].*

N.B: The vascular hyperpermeability first reaches the large superficial vessels and then progress to the capillaries of the external plexiform in the first 2 months of the installation of diabetes [2]. Vascular drop comes in response to the reduced surrounding metabolic needs of the neuroretina (apoptosis of the neuroretina being the first event of DR), so it is conceivable that vascular apoptosis represents a final response to local cell death in the retinal sensory tissue.

Diabetes increases the permeability of EC monolayers by activating the uPA/uPAR system. This system catalyzes the conversion of plasminogen to plasmin. Plasmin can degrade the extracellular matrix (ECM), activate latent TGF-β, and convert inactive pro-MMPs (matrix metalloproteinases) such as MMP2 and MMP9 into their active form (Figure 15). Those MMPs repress the PEDF (Pigment Epithelium Derived Factor) breaking the balance of PEDF and VEGF that is critical for preserving retinal vascular barriers. PEDF is multifunctional and acts on a variety of cell types by its different molecular domains: it is known for its neuroprotective properties (anti-permeability, by its residues 78-121), anti-angiogenic (thanks to its residues 24-57), anti-apoptotic (by its residues 78-94) anti-oxidative and anti-inflammatory, and neuronal differentiation (residues 58-101) [19,20]). Dysregulation of the cellular function of the PE is involved in DR through the production of inflammatory cytokines and the apoptotic and inflammatory pathways mediated in diabetes by caspase-14.

Caspase-14 breaks the cellular function of the PE barrier (Figure 16) [22]. This disorganization of the cytoskeleton is at the basis of the hyper-permeability of the PE cells and the dysfunction of the epithelial barrier at the origin of the DME [22].

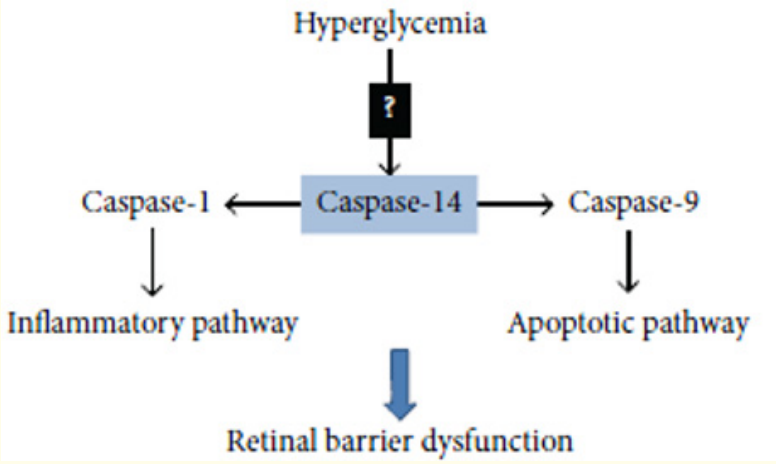


Figure 16: *Pathway of apoptosis induction and inflammation of the retinal vascular barrier by the pigment epithelium [22].*

Thus, the affectation of the epithelium membrane initiates not only the progression of neovascularization, but also microangiopathy and DME [21].

Apoptosis of retinal sensoriel cells

Diabetes increases apoptosis in amacrine glial cells (Muller cells and astrocytes) especially in the internal retina where ganglion cells are found [2]. The total number of apoptotic cells in diabetes is 10 times higher than the number reported for vascular cells. Indeed, atrophy of nerve cells is the very first event of DR, even before the formation of microaneurysms and can occur independently of vascular stress. In diabetic eyes, the reduction in the thickness of the retinal nerve fiber is detected even without retinopathy. These structural deteriorations are associated with functional deficits (decrease of the amplitude in the electroretinogram and loss of contrast sensitivity). The rate of neural apoptosis remains constant throughout diabetes and elevation of intraocular pressure aggravates this effect (posterior vitreous detachment, as in the case of strong myopia, would avoid this disadvantage) [2].

Excitotoxicity of Glutamate (Figure 17) [2] and the loss of the trophic support/survival signaling are the potential causes of retinal cells apoptosis in diabetes; Diabetes depletes BDNF (brain-derived neurotrophic factor) content in the retina, increasing the dopaminergic loss of amacrine cells.

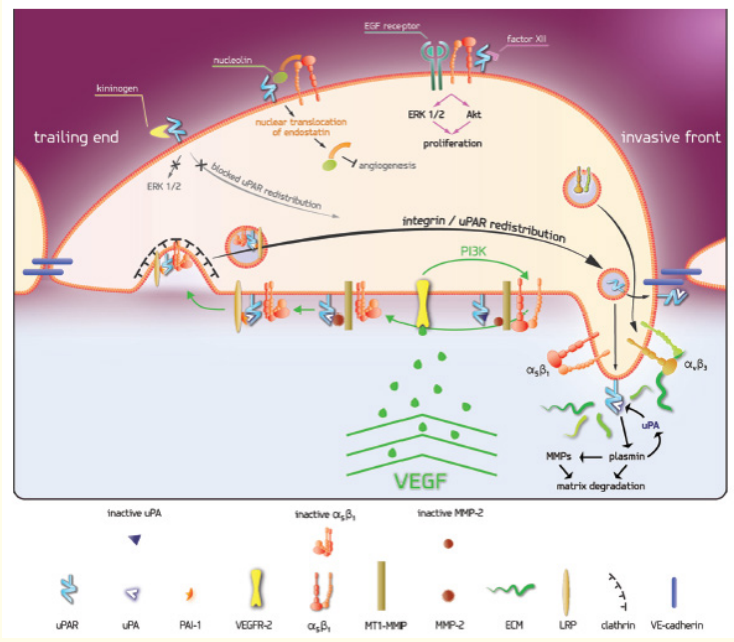


Figure 17: *Mechanisms of permeabilization and of angiogenesis [30].*

Thickening of the basal membrane and vitreomacular traction (TVM)

Müller cells and astrocytes secrete factors that induce the formation of tight junctions in the retinal vasculature. Diabetes affects this property of maintaining the barrier. Under diabetes, Müller cells significantly increase the production of glial fibrillary acidic proteins, contributing to the stiffening of basal vascular membranes; In the diffuse DME, the PE overexpresses the rigidifying cytokeratin.

The vitreomacular interface whose junctions are altered by the cytokines in the vitreous: allows exacerbation, favoring a DME. The vitreous cortex thickens due to infiltration of glial and inflammatory cells. The posterior vitreous cortex thickened and stretched exerting horizontal and vertical traction contributes to the formation of tense membrane in the macula of diabetics. It adheres to the internal limiting membrane of the diabetic and causes traction and macular thickening.

The accumulation of AGEs in the vitreous and retina increases the cross-linking of collagen with other adhesion molecules of the posterior vitreous cortex and the internal limiting membrane (ILM), which reinforces the adhesion of posterior vitreous cortex to ILM and strengthens persistent vitreomacular traction (TVM) even if changes in the vitreous promote posterior vitreous detachment (PVD), hence the progression of the DME.

Implication of human stem cells (HSC) and the bone marrow (BM)

A newly identified BM-derived protein, BMP2 (bone morphogenetic Protein2), with angiogenic and inflammatory properties, is overexpressed by retinal ECs in diabetes [23]. It significantly induces VEGF synthesis by Muller cells in a dose-response effect on

the permeability. At the same time, the pro-inflammatory effects of BMP2 are manifested by the induction of ICAM-1, which recruits leukocytes to adhere to ECs and overexpress IL-6 and IL-8 interleukins [23].

Diabetes produces chronic inflammation. To look for the origin of this chronicity, it is necessary to look at the source, which, once affected, starts to generate modified biological and cellular material: The bone marrow (BM). Indeed, diabetes induces a microvascular depletion with low perfusion resulting in a weakening of the hematopoietic fraction and a greater accumulation of fat in the BM. Stem cells are reduced at hypoperfused sites [24]. There is a reduction in colony regeneration by multipotent progenitor cells but not by LSK lineage progenitor cells. These cells have self-renewing capabilities and generate multipotent, life-renewing LT-HSC (Long-term reconstituting Human Stem Cells) stem cells, from which multipotent ST-HSCs (Short-term HSC) are derived, but without the ability to self-renewal. In the BM of diabetes, a higher number of LSK cells with an intact repopulation potential have been found, but it is their mobilization that is inhibited. A substantial defect in CXCL12 inhibition mediated by adrenergic stimulation in mesenchymal stem cells exclusive to the perivascular space also occurs. Hyperglycemia induces neutrophils to secrete non-AGE ligands for AGE receptors (RAGE) and the interaction of these molecules with RAGE on common progenitor/myelogenous cells results in increased myelocytosis.

In response to hyperglycemia, BM secretes iNOS (inducible lithium synthase), PARP-1 (poly ADP-ribose polymerase-1), major mediators of inflammation, capillary degeneration, superoxide production, expression of pro-inflammatory genes and leukostasis associated with diabetes. MO Knock-Out transplantation of PARP-1 or iNOS would therefore be an effective therapeutic approach.

In addition, lipid mediators such as leukotrienes and prostanoids, secreted locally by leukocytes, contribute to the degeneration of retinal capillaries.

Leukostasis results mainly from an increase in monocytes (CD11b) (TNF-α secretors among others) but not granulocytes (Gr1) or lymphocytes (CD3) [24]. Neutrophils also, secreting ICAM-1 play an important role in DR.

Thus, hematopoietic BM-derived stem cells play a central role in the pathogenesis of early DR.

In summary, hyperglycemia directly compromises the function of the BM cells and causes a lack of mobilization of hematopoietic stem cells depending on the microenvironment (niche) of stem cells. These hematopoietic cells dysregulated quantitatively and qualitatively migrate to organs affected by diabetes, initiating inflammation, cell dysfunction and accelerated apoptosis (Figure 18) [24].

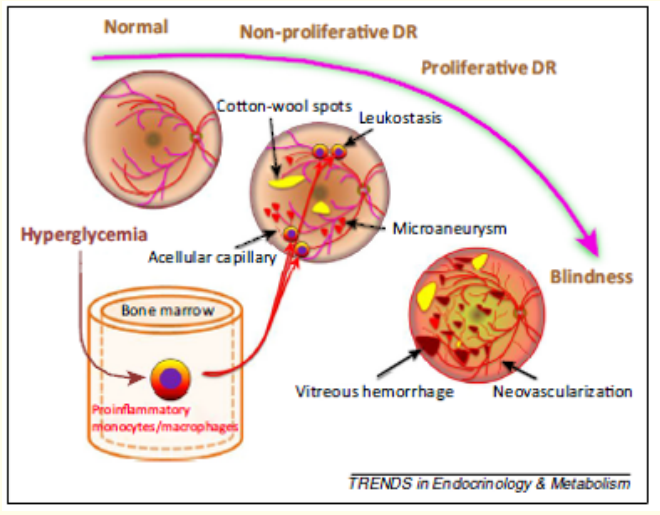


Figure 18: *Role of BM affectation by diabetes in diabetic retinopathy [24].*

Responders vs Non responders to the treatment

Differential analysis of the systemic gene expression pattern of DME patients who responded to anti-VEGF treatment and patients who did not respond to treatment showed that it is the number of extracellular matrix receptor genes expressed and the number of cell adhesion molecule genes expressed that showed differences between the two groups: Deregulation of the Wortmanin/b-catenin signaling pathway that complicates diabetes of retinal inflammation, vascular leakage and neovascularization, in parallel with the regulators apoptotic are the most decisive in the differentiation between responders and non-responders. The total number of overexpressed genes was 5 while 105 genes were repressed. None of the 5 overexpressed genes were common between responders and non-responders. 15 genes of adhesion molecules expressed in responders against 26 in nonresponders. Expression of IL-8 is 6-fold higher in non-responders [25].

In non-responders, there was greater expression of genes for angiogenesis, receptor activity, transcription factors, and stress-related genes. 63 genes overexpressed, against 50 repressed genes of the metabolic pathway. In the responders, 20 genes are overexpressed, and 40 of repressed [25].

In the inflammatory pathway, 18 genes are found overexpressed and 8 repressed in diabetics vs. nondiabetics. TGF-β which is involved in cellular processes and survival in normal and unhealthy conditions was inhibited in responders but not in non-responders. This would indicate the need to combine anti-VEGF treatment with anti-TGF-β in non-responder DMEs [25].

In non-responders, VEGF receptors (kinase insert domain receptor-KDR) are less expressed! This suggests that there is another route than that of VEGF. Indeed, the ephrin receptor pathway and FT families such as FOX and HOX were overexpressed in comparison with responders. In responders, anti-VEGF post-treatment restricted KDR (VEGFRecptor2) expression [25].

Hyperglycemia stimulates also semaphorin 3A, ligand of neuropilin1 (nrp1), an analogue to VEGF [31]. Nrp1 can fix at the same time VEGF and sema3A on their site on nrp1. Then phosphorylation of Src, FAK, VA-cadherin, which collapse the cytoskeleton, increases the permeability and accelerates the breakdown of the retinal vascular barrier. On the other hand, nrp1 promotes the binding of VEGF on its VEGFR2 receptor [31]. The latter activates the angiogenesis pathway and the proliferation of the PE and the migration of monocytes and inflammatory molecules (4EBPI, S6K) and further activates the synthesis of VEGF [26,27]. N.B: Sema3A is expressed early and significantly from the first 12 weeks of diabetes onset while VEGF expression is only mild. It was not until week 14 that VEGF reached its peak of expression and exceeded the rate of sema3A which is constant from the start.

Taking in charge of the DME

- Close control of systemic factors: blood glucose, blood pressure, lipids. An HbA1c level of 7% and a systolic blood pressure < 140 mmHg should be targeted. Attention, a level of HBA1c ≤ 6% can cause cardiovascular mortality. The addition of fenofibrates should be considered. It is important to administer glitazone discontinuously to avoid the risk of fluid retention. Laser treatment can elevate intraocular oxygen tension, activate phagocytosis with pigment epithelial and glial cells, and reduce the production of vasoactive cytokines (VEGF). However, the laser causes sub-retinal fibrosis and enlargement of the scars. The focal/grilled laser is now limited to patients with DME that does not involve fovea. The addition of ranibizumab with the laser worsens the vision with risk of vision loss due to thermal complications. Pigment epithelial cells at the margins of burns modulate several cytokines via photoreceptors. One way to maximize efficiency is to apply subthermal selective intensity to the PE and use an 810 nm diode laser to reduce burn intensity and avoid macular chromophore uptake; the micropoluse techniques increase the delays between pulses and reduces the size of the retinal lesions by eliminating heat diffusion and development of the post-treatment lesion.
- PPV + ILM: The effective method that preserves the PE, improves oxygenation and releases traction of the macula, reduces thickness, reduces ischemia, removes cytokines and vitreous AGEs (which are 10 to 20 times more abundant) retina and decreases vasopermeability; results in only 38% improvement in visual acuity by 10 letters. Intraoperative administration of anti-VEGF agents and corticosteroids could further improve post-operative outcomes of treatment (Figure 19) [21].

- Microplasmin causes vitreolysis, releasing vitreomacular adhesion in DME only. A complete PVD appeared in 38% of the DMEs (24 eyes) after 1 injection of plasmin and the total increases to 51% after the second injection. The central macular thickness improves in 100% of cases and the BVCA in 89% of cases [33].

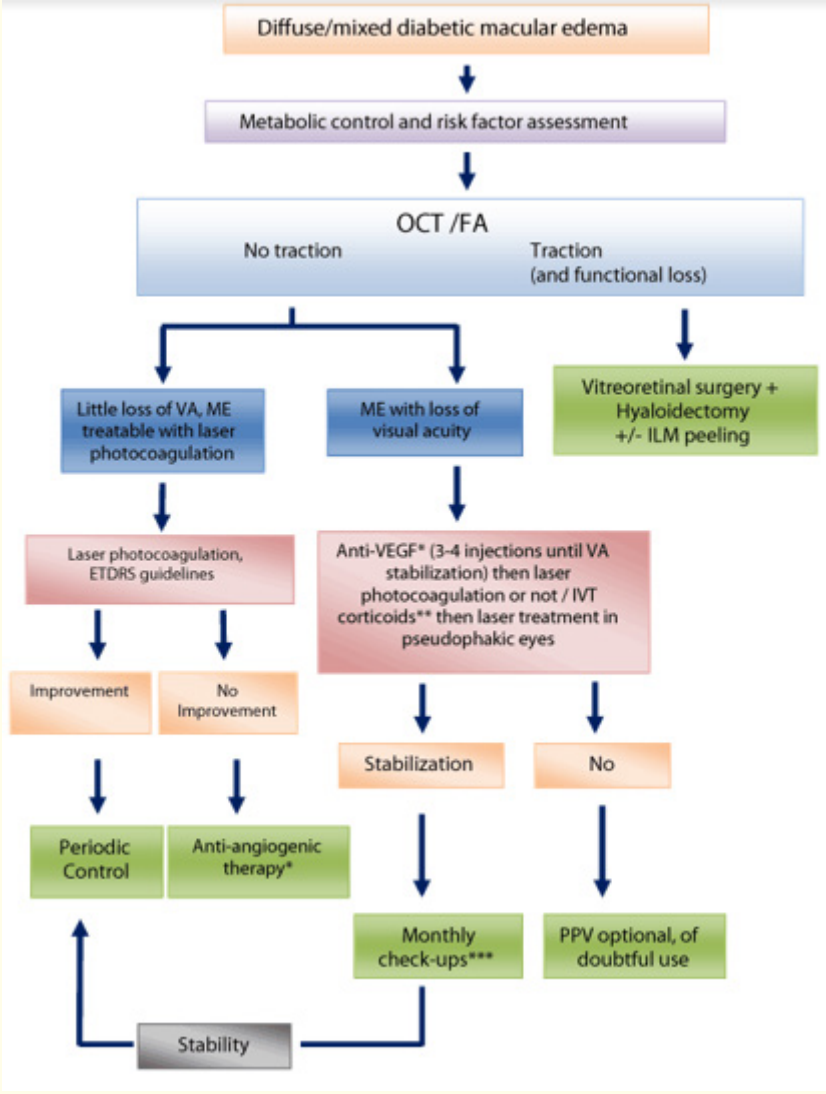


Figure 19: *Algorithm of treatment of large DME.*

Treatments

Recent and old studies focus on VEGF as a mediator of increased permeability and several clinical studies target VEGF for the treatment of DME. However, the targeting of this molecule seems to have limitations (temporary improvement and recurrence of edema in the majority of patients a few weeks after treatment). It does not eliminate hypoxia and requires repeated administrations. However, care must be taken because VEGF does not only regulate angiogenesis but also maintains and differentiates mature choriocapillaries. As a result, the delivery of these Anti-VEGFs must be specific to neovascularization sites or limited to short duration.

In the DRCR (Diabetic Retinopathy Clinical Research) study (Protocol 1), the DME persisted in 50% of patients, even 1 year after monthly injections of anti-VEGF, ranibizumab. This suggests targeting other molecules or mechanisms that operate independently or in conjunction with VEGF in the pathogenesis of this disease.

The most important treatment emerging for DME therapy nowadays include corticosteroids, VEGF inhibitors, PKC inhibitors, RNA small interfering (si-RNA), hydroxy-3-methyl inhibitors -glutaryl coenzyme A reductase and non-hormonal anti-inflammatory agents [32] (Figure 20).

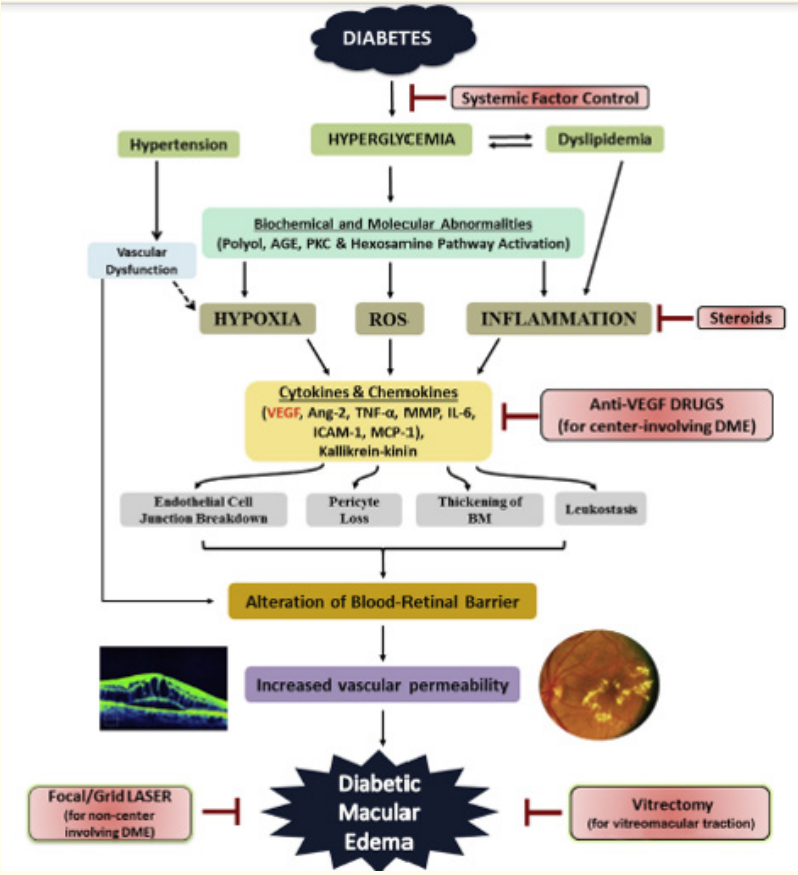


Figure 20: *Stages of formation of the DME and corresponding treatments [32].*

Any molecule overexpressed or inhibited directly or indirectly by hyperglycemia and/or diabetes may be targeted in an attempt to restore normal homeostasis.

Others molecules are certainly currently under development in pharmacotherapy and anti-inflammatory molecules [36].

Understanding the cellular and molecular dynamics of pathological changes in DR is important for identifying therapeutic targets and developing better modalities in conjunction with glycemic control to protect the retina against chronic insults that accumulate and cause persistent and severe stress exceeding the capabilities of coping mechanisms.

Conclusion

This review herein expands on the pathophysiological mechanisms underlying the DME, as well as the new therapeutic approaches currently under development or to be developed in order to deduce, among a great variability, the most effective indication for each patient individually, taking into account his genetic and immune profile.

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