

A basic scientific understanding of diabetic retinopathy

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Introduction

Diabetic retinopathy (DR) is classified into nonproliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR), with the latter identified by the growth of new retinal blood vessels. In this chapter, we will review our current understanding on the pathophysiology of DR.

One of the earliest changes seen in DR is thickening of the vascular basement membrane. Basement membrane thickening is thought to be a result of the upregulation of basement membrane proteins or from reduced proteolytic activity. The basement membrane provides support for both endothelial cells and pericytes and this change in the basement membrane can lead to pericyte and endothelial cell damage. Retinal vascular pericytes form the blood–retinal barrier and, thus, disruption of vascular pericytes leads to leakage of proteins and fluid into the retina. Continued loss of pericytes eventually results in complete loss of the capillary network and retinal ischemia. In addition to these microvascular changes, there is mounting evidence that retinal neuronal and glial abnormalities also occur as diabetes progresses. The retinal neurovascular unit consists of retinal neurons, Müller glia, and their vascular supply. Microvascular changes can lead to damage to these neurovascular units, resulting in neuronal degeneration [1].

Although the precise mechanism leading to pericyte loss and neuronal degeneration is not yet known, current evidence supports several key pathways that are thought to be responsible for cellular damage. These different pathways are described in Figure 2.1

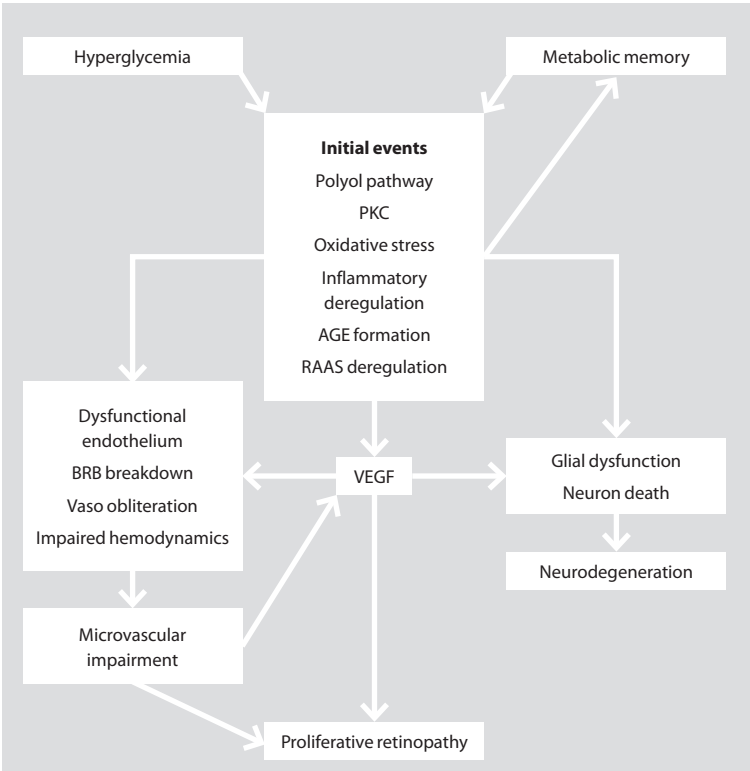


Figure 2.1 Key pathways leading to diabetic retinopathy. Initial events are due to hyperglycemia and include the polyol pathway over action, protein kinase C (PKC) stimulation, increased oxidative stress, amplified inflammatory response, advanced glycation end-product (AGE) formation and renin-angiotensin-aldosterone system (RAAS) dysfunction. 'Metabolic memory' through epigenetic mechanisms propagates many of these processes even after good glycemic control is achieved. These initial events lead to microvascular impairment, and neurodegeneration. The hallmarks of neurodegeneration are neuron death and glial dysfunction. The hallmarks of microvascular impairment are dysfunctional endothelium, blood-retinal barrier (BRB) breakdown, vaso-obliteration, and altered microvascular hemodynamic response. The loss of neuroprotective factors and ischemia trigger vascular endothelial growth factor (VEGF) activation, which has a key role in the disruption of the BRB and neurovascular unit.

Polyol pathway

The polyol pathway (Figure 2.2) metabolizes excess glucose. Within the retina, aldose reductase reduces glucose into sorbitol using nicotinamide adenine dinucleotide phosphate (NADPH) as a cofactor. Sorbitol is then metabolized into fructose by sorbitol dehydrogenase.

The accumulation of intracellular sorbitol is deleterious to the health of retinal cells by several mechanisms. One mechanism is osmotic in nature [2]. In addition, fructose, which is produced in excess by the overactive polyol pathway, can be phosphorylated to fructose-3-phosphate and then metabolized to 3-deoxyglucosone, both of which are effective glycating agents and can result in the production of advanced glycation end-products (AGEs) [3], which are discussed later in the chapter.

The antioxidant capacity of retinal cells may also become reduced by an overactive polyol pathway because NADPH is required for glutathione reductase to regenerate glutathione, a major reducing agent in cells [4]. The aberrant shift of the NADPH:NADP⁺ ratio by sorbitol dehydrogenase has also been proposed to trigger NADPH oxidase, which can lead to the increased production of reactive oxygen species within the cell [5]. Oxidative damage leads to cellular damage and may play a unifying role in the pathogenesis of DR.

In vitro studies demonstrated that increased aldose reductase is localized in several retinal cells including pericytes, retinal endothelial cells, ganglion cells, and Müller cells [6,7]. The accelerated destruction of these cells has been associated with increased aldose reductase activity. Particular attention has been directed at pericytes. Exposure

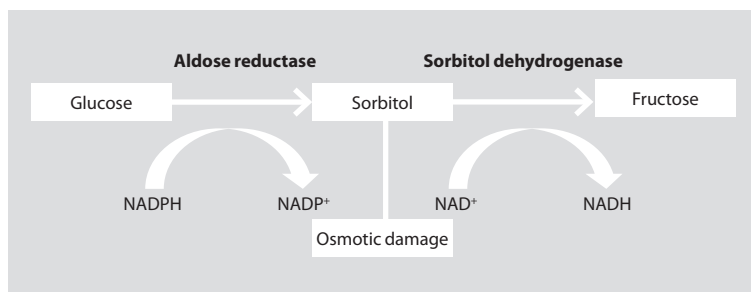


Figure 2.2 The Polyol Pathway. NAD, nicotinamide adenine dinucleotide; NADP, nicotinamide adenine dinucleotide phosphate; NADPH, nicotinamide adenine dinucleotide phosphate (reduced form).

of these cells to increased concentrations of glucose resulted in their reduced viability, an effect that was reversed upon the addition of aldose reductase inhibitor [8]. However, in vivo studies yielded conflicting results. Studies performed in galactose-fed animals showed that aldose reductase inhibitors were able to reduce the incidence and severity of diabetic retinal lesions. However, long-term studies demonstrated that aldose reductase inhibitors were not able to prevent vascular damage [9–11]. The polyol pathway and the activity of aldose reductase has also been implicated in causing thickening of the retinal capillary basement membrane, and altering vascular permeability [12,13].

Advanced glycation end-products

The formation of AGEs contributes to development of DR. In diabetes, AGE formation is accelerated because of the increased availability of glucose. Reducing sugars, present in large abundance in diabetes, react with free amino groups of proteins, lipids and nucleic acids forming a Schiff base intermediate which is converted into an AGE [14] (Figure 2.3).

These products are known to exert an effect on cells by several mechanisms. There are a variety of cell-surface receptors for AGE which when activated lead to proliferative, oxidative, and pro-inflammatory events. AGEs can also form crosslinks between proteins found in the basement membrane or extracellular matrix proteins of vessel walls. In fact, the AGE levels within vessel wall proteins have been shown to correlate with those in the serum as well as with severity of retinopathy [15].

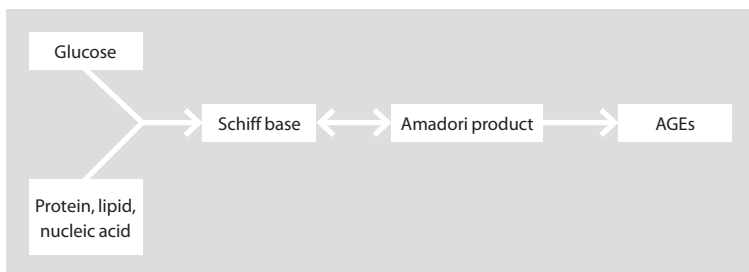


Figure 2.3 Advanced glycation end-product (AGE) formation. Reducing sugars react with proteins, lipids, or nucleic acids to form a Schiff base, which is converted into Amadori products and eventually advanced glycation end-products.

Finally, AGEs and their intermediates can form oxidative products that increase the oxidative stress placed on retinal neurons.

In vivo studies support the role of AGEs in the pathogenesis of diabetic retinopathy. Animals exposed to different AGE inhibitors such as amino-guanidine hydrochloride [16] and pyridoxamine [17] had a reduction in structural abnormalities seen in experimental models of DR.

Protein kinase C activation

Protein kinase C (PKC) is a serine-threonine kinase involved in a large array of signal transduction events responding to specific hormonal, neuronal, and growth factor stimuli, including vascular endothelial growth factor (VEGF). There are several isoforms, the most relevant to DR being $\beta 1$ and $\beta 2$. Much like G-proteins, in which a relatively limited signal transduction language leads to a wide array of cell-specific responses, PKC is ubiquitous, but its activation leads to different actions in different cells. Hyperglycemia stimulates the de novo synthesis of diacyl glycerol (DAG), which is a potent activator of PKC [18]. PKC activation results in several downstream effects including increased vascular endothelium permeability, altered retinal hemodynamics, increased expression of basement matrix proteins, angiogenesis, cytokine activation, and locally increased inflammation and leukostasis [19]. These changes all contribute to the pathogenesis of diabetic retinopathy.

VEGF-mediated activation of PKC has been shown to alter tight junctions in retinal endothelial cells, promoting vascular permeability by phosphorylation of occludin [20]. Phosphorylated occludin becomes internalized into endosomes, resulting in a breakdown of the blood–retinal barrier. This leads to disruption of the neurovascular unit in the retina. The neurovascular unit refers to the close association and interdependency of neurons, glia, and vasculature in the retina and brain. This association allows for neurotransmitter regulation, removal of metabolites, and energy homeostasis. The neurovascular unit is dependent on the blood–retinal barrier and breakdown of this barrier results in abnormal retinal function [21].

Hemodynamic changes and RAAS system

It has long been known, beginning with the Wisconsin Epidemiological Study of Diabetic Retinopathy (WESDR) and continuing with the UK Prospective Diabetes Study (UKPDS), that blood pressure plays a significant role in the progression of DR [22]. Hypertension is thought to contribute to the progression of DR through two mechanisms. One, the local mechanical stress (thought to lead to endothelial dysfunction within retinal vessels) [23]. Two, the renin-angiotensin-aldosterone system (RAAS), systemically deregulated in diabetes and independently implicated in the DR pathogenesis.

Renin, angiotensin-converting-enzyme (ACE)–I and -II, and angiotensin receptor levels are reported to increase in the retina during PDR [24]; ACE inhibition has been shown to hamper retinal neovascularization in experimental models [25]. The Diabetic Retinopathy Candesartan Trials and Renin Angiotensin System Study both reported a reduction in the incidence of retinopathy in Type 1 diabetes at baseline, as well as a reduction in the progression of retinopathy, when ACE inhibitors were utilized [25,26]. The precise mechanism by which RAAS contributes to DR is not well elucidated.

Inflammation

In diabetes, there is a subclinical increase in the overall inflammatory state that leads to the development and progression of diabetic complications. In the retinal vasculature, this results in increased leukostasis and VEGF-mediated vascular permeability. The extent of this disruption leads to variable capillary nonperfusion influencing DR progression [27].

There is a significant increase in the expression of proinflammatory chemokines, cytokines, and surface adhesion molecules such as interleukin-1b, tumor necrosis factor- α , inducible nitric oxide synthase, cyclo-oxygenase-2, VEGF, intercellular adhesion molecule 1, and integrins. The extent of this deregulation has been correlated with the progression of DR.

Perhaps the most notable aspect is the synergy between endothelial dysfunction and increased surface adhesion molecules in promoting

leukocytosis [27]. Adhesion of leukocytes to the vascular walls leads to increased expression of cytokines and growth factors resulting in microglial cell activation, mobilization of neutrophils and monocytes, and increased vascular permeability [28]. The potent effects of intravitreal steroids in reducing diabetic macular edema support an inflammatory component beyond the anti-VEGF effects of steroids. Epigenetic modifications may also control the expression of proinflammatory molecules by activation of the proinflammatory transcription factor NF- κ B. These changes result in a prolonged inflammatory state that lasts beyond the period of poor glycemic control.

Vascular endothelial growth factor

The most widely studied growth factor in the development of DR is VEGF. It is a growth factor that promotes angiogenesis, causes breakdown of the blood–retinal barrier, stimulates endothelial proliferation and increases vascular permeability in response to hypoxia. The 165 AA isoform has particular implications in the development and progression of DR. Cellular functions of VEGF are mediated through two membrane-bound tyrosine kinase receptors (VEGFR-1 and VEGFR-2). VEGFR-2 is expressed on vascular endothelial cells and is the receptor that is mostly responsible for angiogenesis. Activation of VEGFR-2 in PDR increases endothelial cell permeability, stimulates cellular migration, survival and proliferation, and leads to neovascularization [29]. Much of the downstream regulatory effects of VEGF can be explained by the activation of three separate pathways, although other pathways may play a role. The phosphatidylinositol 3'-kinase/Akt signaling pathway promotes endothelial cell survival [30]; the Erk 1/2 pathway promotes cellular proliferation [31]; the PKC pathway increases vascular permeability.

VEGF production is induced in cells that are not receiving enough oxygen [32]. When a cell is deficient in oxygen, it produces hypoxia-inducible factor, which stimulates the release of VEGF. Thus, VEGF-mediated damage may be a late event as obliteration of the retinal capillary networks ensue. The central role of VEGF in the pathogenesis of DR is further supported by the successful clinical use of anti-VEGF

agents such as bevacizumab, ranibizumab, and aflibercept for the treatment of diabetic macular edema [33]. Treatments will be discussed in more detail in Chapter 8.

Reactive oxygen species

Oxidative stress is defined as an aberrant increase in the production of reactive oxygen species (ROS) or a corresponding decrease in the capability of a cell to neutralize the ROS using antioxidants. The resulting oxidative stress causes damage to cellular structure and may also induce epigenetic changes. Diabetes is associated with increased production of ROS through multiple mechanisms including the activation of the polyol pathway, formation of AGEs, and increased inflammation. It is also associated with a decrease in the level of antioxidant enzymes such as glutathione through the increased demand for NADPH. Interestingly, oxidative stress has been proposed to be a unifying mechanism linking the various biochemical pathways important in development of DR. In this model, hyperglycemia-induced ROS production is an upstream event which causes the inhibition of the key glycolytic enzyme glyceraldehyde-3 phosphate dehydrogenase (GADPH). Inhibition of GADPH results in the accumulation of glycolytic intermediates which activates the polyol, AGE, and PKC pathways [34].

Epigenetics

Epigenetics is the study of heritable changes in gene expression that occur without changes in DNA sequence. It is a broad reaching term, but encompasses the control of gene expression mediated by the remodeling of chromatin, the basic storage unit of DNA. Chromatin is constructed from repeating nucleosomes, a structure composed of DNA wrapped around a histone octamer. Epigenetic control is mediated through the recruitment of enzymatic complexes that alter chromatin structure by covalently modifying the histones. An 'open' chromatin conformation confers accessibility of transcription factors to the DNA, allowing for gene transcription, while a closed chromatin structure prevents access [35].

The roles of epigenetic modifications in DR are beginning to be understood. Hyperglycemia leads to activation of the AGE, PKC, and

ROS pathways. All of these pathways are capable of inducing changes to histones such as histone methylation. For example, hyperglycemia-induced ROS formation leads to epigenetic histone modifications in the promotor of NF- κ B subunit p65. Methylation of histone H3 at lysine 4 and demethylation of histone H3 at lysine 9 acts to prolong the expression of p65. These histone modifications are long lasting and despite normalized glycemic control, increased expression of p65 persists. Expression of p65 leads to upregulation of proinflammatory genes, contributing to the inflammatory response seen in DR [36].

Instituting tight glycemic control in previously poorly controlled diabetic patients does not immediately slow the progression of DR. An expanding body of literature has introduced the concept of ‘metabolic memory’ attributed to epigenetic modifications to help explain this phenomenon (Figure 2.4).

Other pathways affecting histone acetylation and repression of gene expression by microRNAs have been implicated in affecting metabolic memory in DR. Together, the concept of epigenetic modifications may represent a novel pathway for potential therapeutic intervention.

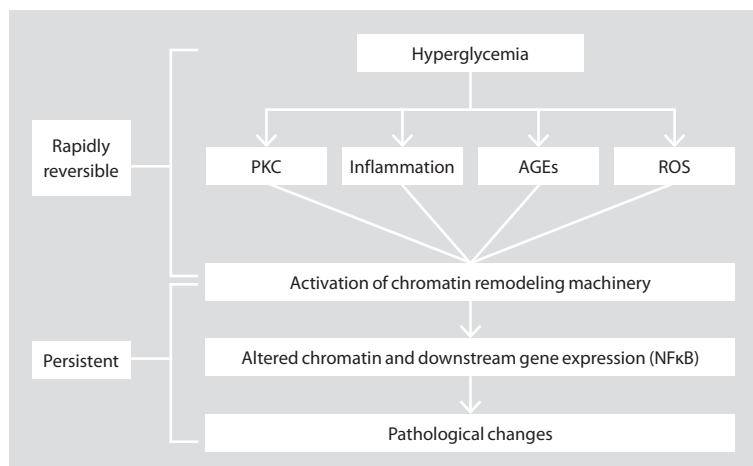


Figure 2.4 Epigenetic changes lead to persistent gene expression alterations, even after the institution of good glycemic control. AGE, advanced glycation end-product; PKC, protein kinase C; ROS, reactive oxygen species.

Conclusion

Diabetic retinopathy remains a leading cause of visual impairment in the world. Although it has been extensively studied, a complete understanding of the pathophysiology remains to be described. Multiple pathways are involved in the pathogenesis of this complex disease process. Although unifying themes are beginning to emerge such as the role of epigenetics and oxidative stress, it is likely that successful treatment of DR will involve targeting multiple pathways. A basic science understanding of the pathogenesis of DR will enable us to better apply our current treatments and help shape the future direction of research to prevent complications from this devastating disease.

Key take-home messages:

- There are various pathways leading to the histopathologic changes seen in diabetic retinopathy.
- ‘Metabolic memory’ and epigenetic modifications play a role in the pathogenesis of diabetic retinopathy.
- Anti-VEGF agents can reduce diabetic macular edema and the progression of diabetic retinopathy.
- Oxidative stress, epigenetic modifications, and inflammation may be unifying mechanisms for the development of diabetic retinopathy.

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