Review Article

Diabetic Retinopathy: A Microvascular but also Neural Disorder

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INTRODUCTION

Diabetes mellitus is a systemic metabolic disorder, the incidence of which is alarmingly increasing in the US. Multiple etiologies are involved in the development of diabetes resulting primarily in hyperglycemia and other metabolic alterations including dyslipidemia and protein uncouplings. Classified as type I and II according to its pathogenesis, both types present with similar symptomatology and complications. In this systemic disorder there are neural and microvascular complications that include the retina, peripheral capillary perfusion, and the kidney. Approximately one third of diabetic patients will eventually develop some degree of diabetic retinopathy (DR), which has become the most common cause of new cases of blindness in US in patients ages 20 to 74 [1]. Thus, in this review the pathogenesis of microvascular and neural complications associated with diabetic retinopathy as well as current and promising new therapies that are arising based on these pathogenetic factors are discussed.

MICROVASCULAR DISORDER

Diabetic retinopathy was previously considered an exclusively microvascular disorder. Vascular damage is primarily due to increase in permeability and a decrease in perfusion including lesions in the basement membrane of retinal capillaries, selective loss of endothelial cells and pericytes, microaneurysm formation and leakage, but also macular edema, and retinal ischemia which leads to hypoxia-induced proliferative angiogenesis and cotton wool spot formation. The initiation factor of vascular injury seems to be elevated levels of vascular endothelial growth factor (VEGF). VEGF-A signals mainly through the VEGF receptor 2 on endothelial cells, leading to increased vascular permeability, angiogenesis, and endothelial cell proliferation [2]; however, several isoforms of VEGF- A with different functions can be expressed. In the diabetic retina, VEGF-A$_{165}$a and VEGF-A$_{165}$b exert opposite effects on both vascular permeability and angiogenesis. In contrast to VEGF-A$_{165}$a, the VEGF-A$_{165}$b isoform does not stimulate but rather inhibits vascular permeability and angiogenesis [3]. The effects of VEGF-A$_{165}$b are achieved by interfering with VEGF-A$_{165}$a actions on endothelial cells. Thus the down regulation of VEGF-A$_{165}$b in DR [4] may explain the increased permeability and angiogenesis. The properties of VEGF made it an excellent target for DR therapy. In the last decade, blockade of VEGF actions by using anti-VEGF antibodies, inhibitors of VEGF intracellular transcription and signaling cascade, inhibitors of extracellular VEGF and VEGF receptors (bevacizumab, ranibizumab, bevasiranib, midostaurin, pegaptanib, and aflibercept) have resulted to be effective antioangiogenic drugs improving visual function in age-related macular degeneration and more recently in diabetic macular edema.

Another factor involved in VEGF activation in diabetes is hyperglycemia-induced activation of protein kinase C (PKC) in the retina via the diacylglycerol pathway. Novel alternative therapies to VEGF inhibition for DR include the use of systemic (oral) PKC beta inhibitors, which can that attenuate visual loss [5]; however, results from clinical trials are not consistent and currently is still pending FDA approval. While some studies suggest moderate improvement in visual loss by a 40% and no effect in progression of DR [6], later studies by the same group stated a decrease in the progression of macular edema [7]. Subclinical inflammation is another factor recently shown to stimulate VEGF-induced permeability in DR [8], thus currently clinical trials with intraocular anti-inflammatory drugs are in progress as potential therapies for DR, targeting mainly NF-KB, and TNF-alpha signaling [9].

One more factor to consider is the direct stimulatory effects of Angiotensin II in the secretion of VEGF observed in cultured smooth muscle cells [10]. In the RAS Study trial (RASS) angiotensin converting enzyme inhibition as well as the inhibition of angiotensin II receptors with losartan delayed the progression of DR in normotensive patients [11]. Because of the high prevalence of hypertension in diabetic patients, blockade of the renin angiotensin system (RAS) could have in addition to the antihypertensive effects, beneficial effects in DR independent of the decreases in blood pressure.

It is also well-known that oxidative stress is elevated during the progression and complications of diabetes and therefore in DR [12]. Free radicals such as superoxide anion are elevated and the level of antioxidant enzymes is decreased. But as in other systemic disorders where oxidative stress and free radicals are

elevated the effects of antioxidants on DR are controversial. The only antioxidant treatment proven to have an effect on attenuating the progression of DR is high-dose of vitamin-E [13].

NEURONAL DISORDER

In addition to the vascular component, current evidence supports a neuronal component in the pathogenesis of DR. Neuronal damage in diabetes is characterized by apoptosis with elevated levels of the pro-apoptotic protein Bax and caspases found in diabetic retinas [14,15]. All neuronal cell types in the retina are susceptible to hyperglycemia-induced apoptosis [16] including ganglion, amacrine, horizontal, and photoreceptor cells, but ganglion cells (RGC) seem to be the most sensitive. Changes in neural function have been detected in several studies using full field and multifocal electroretinogram, contrast sensitivity measurement and assessments of color vision [17]. Apoptosis in DR can be induced by several factors such as VEGF, oxidative stress, glutamate and nitric oxide[18-20]

The level of glutamate, a major excitatory neurotransmitter, is also markedly increased in the vitreous of patients with DR and has been associated with excitotoxicity[18]. The glutamate–aspartate transporter (GLAST) removes glutamate from the synaptic clefts thereby stopping the synaptic process. This is a critical function not only for proper neural circuit operation but also to protect neurons against excitotoxicity, as inhibition of this transporter induces RGC death [21]. Upregulation of GLAST expression using glial cell line–derived neurotrophic factor (GDNF) prevents neurodegeneration in the outer and inner retina in STZ-induced diabetic rats [22]. Thus, alterations of both the levels and handling of the major excitatory neurotransmitter of the retina during diabetes may play an important role in diabetic neuronal damage.

Oxidative stress is known to lead to cell death via apoptotic means, which has been demonstrated in DR, particularly in RGCs. Sources of reactive oxygen species (ROS) include but are not limited to NADPH oxidase, mitochondrial electron transport chain, advancedglycation end products (AGEs), aldose reductase/polyol pathway, PKC activation and uncoupling of endothelial nitric oxide synthase (eNOS). Some of these components, such as PKC have also been involved in the vascular pathogenesis of DR. Glutathione (GSH) is an endogenous antioxidant that exhibits an inverse relation with HbA1c and which depletion also causes RGC death. In addition, other antioxidants such as specific flavonoids have been proposed to protect retinal ganglion cells from cell death [19].

Nitric oxide (NO) is an important signaling molecule that contributes to neurotransmission, vasodilatation, and immune defense. There are increased levels of NO in diabetic retinas [23] associated with apoptosis. NO synthases (NOS) catalyze the formation of NO. Inducible (iNOS), neuronal (nNOS) and endothelial eNOS are all found in the retina. In a knockout model of iNOS where proliferative ischemic retinopathy was induced, the inner nuclear layer of the central retinal area exhibited fewer areas of apoptosis than in the wild type [20], suggesting a pivotal role for iNOS in ischemia induced apoptosis. NO-mediated neurotoxicity is thought to be mainly mediated by nitrosative stress, which leads to protein nitration, supporting the role of NO in oxidative stress. Furthermore, eNOS polymorphisms found in DR and eNOS uncoupling also yields free radicals in diabetic retina [24].

In addition to its vascular effects, VEGF is a potential neurotrophic factor. In spite of opposite effects on angiogenesis and permeability, both VEGF-A$_{165}$a and b isoforms exert neuroprotective actions in retinal neurons [3]. Several studies have shown that endogenous VEGF is required for proper retinal function and exerts a protective role on RGC, the main target of DR, in various models of neurotoxicity [25]. Thus therapies based on VEGF inhibition would be detrimental for a mainly neural tissue as is the retina.

THERAPEUTIC APPROACH TO DIABETES AS A NEUROVASCULAR DISORDER

Both neural and vascular events occur early in DR but recent studies support that neuronal dysfunction may occur prior to more severe vascular pathologies. Independently of which event occurs first, neuronal disorders have an implication in the vascular complications of DR [26]. Retinal neurodegeneration contributes to the breakdown of the blood-retinal barrier [27] and overexpression of antiapoptotic factors such as Bcl-2 in vascular endothelium inhibits the microvascular lesions of diabetic retinopathy [14].

Despite the increasing incidence, and prevalence of diabetes mellitus worldwide there is no therapy available to prevent or delay the progression of DR once initiated other than intensive lowering of the glycemic levels as shown in two major clinical trials ("The Diabetes Control and Complications Trial" or DCCT and "The United Kingdom Prospective Diabetes Study" or UKPDS). However, elevated glucose is not the only factor involved in DR, low levels of folic acid and elevated levels of homocysteine and glutamate amongst others have been shown to exert a profound impact in the progression of DR [28,29]. Therefore new therapies are being pursued which target these other factors as possible direct mediators of complications in DR.

The therapies currently in use for DR only take into account the microvascular pathogenesis of this disorder but not the neural component. Laser photocoagulation and vitrectomy are the standard therapy indicated in proliferative DR and macular edema. VEGF has shown to exert neuroprotective effects but VEGF blockade is currently used as an additional treatment in proliferative DR or macular edema where the vascular component is the primary target. However by reducing VEGF levels, the normal visual function might be compromised as demonstrated in studies performed in mice where VEGF blockade was found to lead to the death of photoreceptors and Muller glia cells [25,30].

Taking into consideration the neurovascular origin of DR, a potential therapy should include a drug with simultaneously antiangiogenic and neuroprotective properties. Oxidative stress has an impact in both diabetic complications. The fact that antioxidant pathways including the transcription factor NF-E2-related factor 2 (Nrf2) exert a protective effect in type I induced diabetes and diabetic neuropathy [31] make it a good target for a novel therapeutic approach. Nrf2 is only released during stress situations and promotes the transcription of genes involved in

antioxidant defense [32]. If stress is not present Nrf2 is trapped in the cytosol bound to Kelch like-ECH-associated protein 1 (Keap 1). But during oxidative stress it dissociates from Keap 1 and moves into the nucleus where it binds with the antioxidant response element (ARE). GSH is one of the products of this activation. The role of GSH as antioxidant is well characterized, decreasing hyperglycemia-induced oxidative stress and contributing to the activation or production of other antioxidants [33]. Vitamin E and flavonoids which attenuate the progression of DR, have also been shown to regulate Nrf2 signaling. Data from human diabetic retinas shows increased retinal Nrf2 despite low levels of GSH, suggesting that the signaling cascade is impaired in DR. Supporting this notion is evidence that cytosolic Nrf2 and Keap 1 are increased but nuclear Nrf2 is decreased in DR [34]. Therefore strategies utilizing a more targeted approach focusing on Nrf2-Keap 1 could provide a novel therapeutic avenue to enhance antioxidant response and improve retinal dysfunction in DR.

ACKNOWLEDGEMENTS

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Cite this article

Fortepiani LA (2013) Diabetic Retinopathy: A Microvascular but also Neural Disorder. JSM Ophthalmol 1: 1006.