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Review article

Role of oxidative stress, inflammation, hypoxia and angiogenesis in the development of diabetic retinopathy

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Abstract

Diabetic retinopathy (DR) is a retinal disease which is one of the most severe complications occuring due to diabetes mellitus and is a major cause of blindness. Patients who have diabetes mellitus for number of years develop characteristic group of lesions in the retina which leads to Diabetic retinopathy. It is a multifactorial condition occuring due to complex cellular interactions between biochemical and metabolic abnormalities taking place in all retinal cells. Considerable research efforts in the past 20 years have suggested that the microvasculature of the retina responds to hyperglycemia through a number of biochemical changes, which includes polyol pathway, protein kinase C activation, upregulation of advanced glycation end products formation and renin angiotensin system activation. Various previous studies had suggest that interaction of these biochemical changes may cause a cascade of events, such as apoptosis, oxidative stress, inflammation and angiogenesis which can lead to the damage of a diabetic retina, causing DR. This highlights that oxidative stress, inflammation, angiogenesis-related factors triggers the occurrence of retinal complication in diabetes are highlighted.

Keywords: Diabetic retinopathy, Oxidative stress, Inflammation, Angiogenesis

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Introduction

Diabetes is a progressive lifelong disease resulting from body's inability to produce insulin or use it to it's full potential, and is characterized by high circulating glucose in blood. The incidence of diabetes is increasing rapidly and has become one of the main challenges to current health care. In 2013, more than 382 million people worldwide were affected by diabetes, and by year 2035 this number is expected to reach 592 million.[1] Diabetes is the fourth leading death causing disease globally; every 10 s a person dies from diabetes-related causes.[2]. Furthermore, high incidence of undiagnosed diabetes and impaired glucose tolerance also raises concerns for the future. Individuals with impaired glucose tolerance have a greater than 2 times the risk for the development of diabetic complications than individuals with normal glucose tolerance.[3] Diabetes causes vision problems, and diabetic patients develop cataracts at an earlier age, and they are twice as prone to get glaucoma compared with non-diabetic.[4] Over 75% of patients who have had diabetes mellitus for over 20 years will likely develop diabetic retinopathy.[5]

Diabetic retinopathy (DR), a complication of both type1 and type 2 diabetes mellitus is responsible for 4.8% of 37 million cases of blindness worldwide.[6] The World Health Organization (WHO) has DR on its priority list of eye

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conditions which can be partly prevented and treated. No early symptoms are observed in diabetic retinopathy, when visual problem starts to come into view, retinopathy has already advanced to almost a point of no return. Thickening of basement membrane, vascular leakage, apoptosis of retinal cells, angiogenesis, inflammation, neovascularization and traction retinal detachment are characteristic histological features of DR.[7,8] Clinically, DR has been divided into an early, non-proliferative stage (NPDR), and a later, proliferative diabetic retinopathy (PDR). Increase in retinal vascular permeability causes Diabetic macular edema (DME) which is the most common cause of vision loss in NPDR.[9] PDR is the most advanced stage of DR, which is characterized by extensive retinal ischemia, neovascularization due to capillary closure followed by relentless abnormal epiretinal fibrovascular proliferation with tractional retinal detachment and subsequent bleeding. Distortion or detachment of the retina is the main reason for blindness and is due to formation and contraction of fibrovascular epiretinal membrane (ERM). DR is a progressive disease which is caused due to long-term functional and structural impairment of the retina. Complex interactions occuring among biochemical and metabolic abnormalities of retinal cells leads to this multifactorial condition. DR has been usually regarded as microangiopathy of the retina which involves changes in the vascular wall leading to capillary occlusion and thereby retinal ischemia and leakage. And more recently, a number of studies have demonstrated that microangiopathy is only one aspect of a more widespread retinal dysfunction. Upregulated apoptosis of cells in all the layers of the retina has been observed in various animal models of DR and in post-mortem analysis of diabetic retinas from patients.[10] And thus, collective evidence supports the hypothesis that neurodegeneration, together with functional changes in the vasculature, is an important component of diabetic retinopathy.[11] However, the actual interactions of these pathologies to each other and their complete contribution to damage of retina is still quite unclear. Feed forward concept of vascular-neural dysfunction which results in accumulated injury and failing reparative responses which leads to clinically evident features of diabetic retinopathy was proposed by Antonetti et. al.[12]

It is thus evident that pathogenesis of DR is very complex and multifactorial. Acute or chronic exposure to the diabetic milieu results in a range of biochemical and metabolic abnormalities, which while interrelated may also be simultaneously provoked. Considerable research efforts in the past 20 years have implicated various mechanisms including the polyol pathway,[13] protein kinase C pathway (PKC),[14] advanced glycation end products (AGEs) pathway,[15] haemodynamic changes in blood flow[16] and the renin-angiotensin pathway,[17] mitogen activated protein kinase (MAP kinase) pathway,[18,19] the angiogenic pathway[20] and pathways associated with oxidative damage.[21] While the precise pathogenesis of DR remains incompletely understood, oxidative stress, inflammation, hypoxia and related processes are thought to contribute towards DR development.[2,12,22]

Oxidative stress

It has been observed in previous studies that oxidative stress is a common denominator link for the major pathways which are involved in the disease development and progression for diabetic complications. High glucose levels induces intracellular reactive oxygen species (ROS) either directly by glucose metabolism and auto-oxidation or indirectly by forming advanced glycation end products and their receptor binding. Excessive amounts of ROS oxidizes biomolecules, such as, DNA, protein, carbohydrates and lipids after surpassing various endogenous anti-oxidative defensive mechanisms which leads to oxidative stress. Increasing evidence suggests that the damaging effect of elevated glucose in retina may, in part be due to their ability to increase the generation of ROS and lipid peroxides leading to increase oxidative stress culminating in mitochondria dysfunction and apoptosis in retina.[2,22,23] Naruse et al. suggests that DR causes ROS to increase the level of reactive oxygen metabolites.[24] Progression of DR in type 2 diabetes mellitus patients increases catabolites of biomolecules such as nitric oxide (NO), catalase, glutathione peroxide, and lipoperoxide.[25] A number of *in vitro* and *in vivo* studies suggest that oxidative stress is increased in patients with diabetes and animal models of diabetes.[22–26] Hyperglycemia increases the formation of AGEs by increasing non enzymatic glycation, and binding of glycation end products to their receptor resulted in increases the production of intracellular ROS via NADPH oxidase.[22] Furthermore, activation of protein kinase C, via diacylglycerol formation (which is increased in diabetes), results in subsequent ROS production via NADPH oxidase.[27] Furthermore retina has high amount of polyunsaturated fatty acids along with highest oxygen uptake and glucose oxidation relative to any other tissue which makes retina very susceptible to oxidative stress.[28] Thus, increased oxidative stress is a widely accepted participant in the development and progression of diabetic retinopathy.[2,22–25]

Oxidative stress in diabetic retina has the ability to act as a trigger, modulator, and link within the complex web of pathological events that occur in DR. There are various molecular events that underlie and connect the metabolism, inflammation, and the oxidation in DR. Oxidative stress also triggers a series of cellular responses alongwith damaging the functions, which includes the activation of protein kinase C (PKC), JNK stress-associated kinases, and transcription factors including NF-kB, AP-1, p53, HIF-1α, PPAR-γ, β-catenin/Wnt. Activation of these transcription factors can lead to the expression of different genes, involved in functions related to inflammatory cytokines, chemokines, cell cycle regulatory molecules, growth factors, and anti-inflammatory molecules.[29] Variou studies have reported connection between apoptosis and oxidative stress, and in retina, modulation or activation of pro-apoptotic mediators by diabetes-induced ROS has been indicated.[2,19,23] In addition, the role of oxidative stress in diabetic retinopathy is supported by the observations that antioxidants suppress hyperglycemia-induced increased production of mitochondrial superoxide and hightened peroxynitrite levels in the capillary cells of retina which prevent mitochondrial dysfunction and cellular apoptosis in the retina of experimental diabetic animals.[30–32] Antioxidants have also been shown to normalize retinal inflammation in diabetic rat retina.[21,33] Antioxidants usage in DR animal experimental models have proved to be a beneficial therapeutic strategy for the treatment, but more clinical trials data is required on this front.

Inflammation

Inflammation is the innate immune system of body defence where the immune system of the body identifies foreign pathogen or antigen by binding it to specific receptors, this leads to the activation of cytokines production which further induces production of pro-inflammatory mediators.[34,35] Increased production of pro-inflammatory mediators plays important role in DR pathogenesis and inflammation mechanisms make significant contribution towards the development and progression of DR. Many biochemical and pathological retinal abnormalities associated with diabetes are consistent with inflammation.[36] In addition, the gene profile patterns from the diabetic retinas of rodents share similarity with an inflammatory response.[37] Inflammatory response of the retinal vasculature can be triggered by various factors like hyperglycemia, growth factors, advanced glycation end products, elevated levels of circulating or vitreous cytokines and chemokines. Several studies, the overexpression of proinflammatory and proangiogenic molecules was demonstrated in the ocular microenvironment of patients with PDR,[38,39] suggesting that persistent inflammation and neovascularization are critical for PDR initiation and progression. Similar to this there is increased levels of several proinflammatory cytokines in the retina from diabetic rodents.[40–42] Increase in retinal permeability is one of the early pathological sign of retinopathy in experimental diabetes models. The inflammation reaction between ROS and cell adhesion molecules causes breakdown of the BRB and loss of endothelial cells due to leukocyte adhesion, this can be considered as a possible mechanism for diabetes-induced retinal permeability.[36,43] leukocyte adhesion to the microvasculature is one of the earlier events which occurs in diabetic retinal inflammation, this encourages the induction of adhesion molecules such as ICAM-1 and P-selectin, on the endothelium and its leukocyte counter-receptor CD18.[36] Studies have shown that diabetic retinas from mice lacking ICAM-1 and CD18 are protected from the development of vascular permeability, diabetes-induced increase in leukostasis, and retinal capillary degeneration.[36] Regulation of NF-κB, heterodimer with two subunits, p50 and p65, is done by redox control mechanisms, NF-κB transcriptionally activates various cellular genes which are involved in immune response and inflammation.[44,45] Production of proinflammatory cytokines has been associated with IKK-beta/NF-kappa-B pathway, this is a protein network which enhances transcription process of cytokine genes. It is localized in the subretinal membranes and in the microvessels,[46] and is usually stored in its inactive form in the cytosol. Diabetes has been shown to activate NF-κB in rodent retinas,[42,47] and to cause migration of the p65 subunit into nuclei of retinal endothelial cells, ganglion cells, or cells of the inner nuclear layer and pericytes.[48] In addition, it was shown that the NF-κB activity increases in retinal endothelial cells or pericytes exposed to elevated glucose concentration.[49] Hyperglycemia elevates IL-1β in the retina and its capillary cells; Injection of IL-1β into the vitreous of normal rats enhanced retinal capillary cells apoptosis via activating redox-sensitive NF-*k*B in the retina.[50,51] Furthermore, IL-1β, also associated with angiogenesis and increased vascular permeability, accelerates apoptosis of retinal capillary cells through activation of NF-κB, and the process is exacerbated in high-glucose conditions.[52] Anti-inflammatory therapies have prevented DR development as shown in previous experimental models, inflammation suppression and regulation of tight junction proteins by glucocorticoids have also been considered as possible ways to treat or prevent this blindness causing disease.[48,53] Further, the development of retinopathy in diabetic dogs can be inhibited by the administration of an anti-inflammatory compound, aspirin.[54] Various animal model studies has established evidence that anti-inflammatory therapies significantly inhibit development of different aspects of DR.

Retinal hypoxia

Deficits in oxygen delivery to the retina are observed in diabetes,[55] and tissue hypoxia has also been suggested to occur early stage of the disease.[56] A model have been proposed by Curtis et al. to explain how early blood flow changes may contribute to low-grade, chronic inflammation of the retinal vasculature which results in capillary dropout and development of a progressive, irreversible ischemic hypoxia leading to the characteristic microvascular changes.[57] The earliest biochemical and retinal pathology changes appear to begin within first week of the time when the animals becoming a diabetic. These includes formation of AGEs, overproduction of (Vascular endothelial growth factor) VEGF and its mRNA, also consequent leakage of capillary endothelial cells which cause hypoxia in the retina.[58] Diabetes induced hypoxia retinal tissues stimulate angiogenesis by modulating the balance of pro-angiogenic and anti-angiogenic mediators this leads to retinal neovascularization.[59,60] Many of the effects of hypoxia are mediated by hypoxia-inducible factor-1alpha (HIF-1a), an oxygen-sensitive transcription factor.[61] Various hypoxia-regulated cytokines and growth factors are involved in the pathogenesis of retinal neovascularization.[57] Of the vasoactive agents, VEGF, which is also a downstream target of HIF-1a, has received most attention and it is markedly elevated in the vitreous of active proliferative retinopathy patients.[62,63]. Therefore it is necessary to work for a better understanding of the role of hypoxia and its downstream effectors in driving disease progression.

Angiogenesis

New vascular networks develop from preexisting vessels by the process called angiogenesis. Retinal neovascularization is a hallmark feature of PDR and it is characterized by abnormal angiogenesis resulting in abnormal new vessel formation leading to hypoxia and vascular leakage. It has been suggested that metabolic insults to the diabetic retina such as hypoxia may induce retinal inflammation and thereby VEGF expression followed by neovascularization.[64] The vascular endothelium is a dynamic cell layer that sends and receives complex chemical signals to and from other cells in the vessel wall. These signals contribute to monolayer integrity and vascular function.[65] The balance is seriously disrupted in the diabetic retinal microvasculature because of accelerated apoptosis of pericytes and endothelial cells,[66] resulting in progressive vasodegeneration.[67] Damage to the endothelium is accompanied by breakdown of the inner blood–retinal barrier[68] and the local release of growth factors, cytokines

and chemokines, which exert a proangiogenic activity by acting directly on endothelial cells or indirectly by inducing the production of angiogenic growth factors by inflammatory cells. Proangiogenic cytokines, matrix proteins, growth factors, and other important mediators together with deficiency in the production of endogenous angiostatic mediators determine the switch to angiogenesis promotion and progression to sight-threatening diabetic retinopathy.[69] To begin retinal angiogenesis, the pro-angiogenic and anti-angiogenic balance is likely to be shifted such that mitogenic factors are enhanced and/or inhibitory factors are decreased.[59] VEGF is a primary angiogenic factor which mediates ischemia-induced retinal neovascularization, presenting this as an important target for therapeutic intervention in proliferative retinopathies. Vitreous VEGF levels are elevated in patients with PDR.[62,63] The anti-VEGF antibody bevacizumab (Avastin; Genentech/Roche, South San Francisco, CA) is used for diabetic retinopathy treatment, which attempt to abolish these new vessels and/or to prevent their formation.[70–72] While desirable ameliorating effects on neovascularization is observed with VEGF antagonists, long-term effects of repeated intraocular use of such compounds need further studies. It is possible that inhibition of neovascularization under ischemic conditions can serve to promote ongoing ischemia. Additionally, VEGF has a protective effect on other retinal cells, abolishing this physiological function may have adverse side effects.[73,74] More importantly, many problems associated with pathological angiogenesis do not arise directly from the growth of new blood vessels but from abnormalities in the newly developed microvessels, such as increased permeability. Nonetheless, despite its potent anti-VEGF properties, VEGF antagonists may not completely inhibit retinal fibrovascularization. In cases with severe PDR, tractional retinal detachment after intravitreal injection of bevacizumab has been reported previously.[75] It is likely that various other factors participate in the fibrotic and angiogenic processes which are involved in diabetic retinopathy.

Conclusion

Disease development and progression in diabetic retinopathy is caused by multifactorial process which involves various factors beyond VEGF. Inflammation, oxidative stress, angiogenesis and other related pathways modulates the structural and molecular alterations associated with DR. Although the actual sequence of events that culminates in DR development is still not very well understood and is critical to development of remedial therapies.

Conflict of interest

The authors declared that there is no conflict of interest.

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