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The Molecular Pathogenesis of Diabetic Retinopathy - A Spectrum of Pathology Caused by the Disruption of Inner Blood-Retinal Barrier

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1. Introduction

1.1 Epidemiology of diabetic retinopathy

Among diverse microvascular complications of diabetes, diabetic retinopathy is a leading cause of adulthood blindness in the United States. According to the report of Eye Diseases Prevalence Research Group, the estimated incidence of diabetic retinopathy reaches up to 3.4% in US general population (Kempen et al., 2004). The incidence of retinopathy is increasing according to the duration of diabetes. In type I diabetes patients with duration of 20 years or more, the prevalence of any diabetic retinopathy reaches 100% (Klein et al., 1984b). Diverse classification criteria was introduced in diabetic retinopathy, but the presence of retinal new vessel (definition: any new vessels arising from retina or optic disc, extending to the inner surface of retina or into the vitreous cavity) is the most frequently used criterion because of its clinical significance. Panretinal photocoagulation is indicated in proliferative diabetic retinopathy (PDR) with high risk characteristics, where there is a dramatically increased risk of severe visual loss within 2 years (26.2%) compared to that of PDR without high risk characteristics (7%) (The Diabetic Retinopathy Study Research Group [ETDRS], 1987).

1.2 Anatomical and functional changes involved in diabetic retinopathy

The earliest clinical finding in diabetic retinopathy is the presence of microaneurysms and/or retinal dot hemorrhages. Pathologically, thickening of capillary basement membrane, loss of pericytes are early signs of diabetic retinopathy (Cunha-Vaz, 1978; Garner, 1993). Along with perivascular extracellular matrix, pericytes contribute to the stability of retinal microvessels. Pericytes share their basement membrane with retinal endothelial cells and postulated to mechanically stabilize retinal vasculature through N-cadherin-mediated adherence junctions located in peg–socket contacts (Gerhardt & Betsholtz, 2003). Moreover, pericytes communicate with endothelial cell through several

mediators to regulate recruitment and proliferation of pericytes, proliferation of endothelial cells and the functional integrity of blood-retinal barrier. Loss of pericytes is putative cause of microaneurysm formation in diabetic retinopathy. In a more advanced stage, acellular capillaries and vitreo-retinal neovascularization are the characteristic histo-pathologic findings. Loss of retinal capillary cellular components involves both endothelial cells and pericytes. The mechanism of cell loss is to be elucidated, but throughout the diabetic retinal vasculature, increased apoptosis was observed in both animal models and human specimens (Mizutani et al., 1996).

1.3 General patho-physiology of diabetic retinopathy

In non-proliferative diabetic retinopathy, increased vascular permeability and retinal ischemia secondary to retinal capillary drop-out are two major patho-physiologic processes. Proliferation of new vessels and/or fibrous tissue is the hallmark of proliferative diabetic retinopathy. Unlike to normal retinal vessels, newly formed vessels in proliferative diabetic retinopathy are leaky due to the presence of endothelial fenestrae and incompetency of junctions (Wallow & Geldner, 1980; Williams et al., 1988) and usually accompany fibrous proliferation. These features of new vessels are responsible for the aggravation of retinal edema and development of retinal and/or vitreous hemorrhage in PDR patients. Moreover, a contraction of posterior vitreous surface which are adherent to the fibrovascular membrane usually results in the traction retinal detachment. In this chapter, we further discuss about the pathogenesis of diabetic retinopathy in an aspect of blood-retinal barrier dysfunction.

2. Blood-retinal barrier: In health and disease (diabetic retinopathy)

In the mammalian brain, molecular exchange between blood vessel and neuron is tightly regulated by the structure named blood-brain barrier (BBB). Ions, neurotransmitters, macromolecules like plasma proteins, toxins, metabolites and nutrients are regulated for neuronal homeostasis. Several mechanisms are involved in the selective exchange of molecule through BBB. There are two distinctive routes for circulating blood component to reach the central nervous system (CNS): transcellular and paracellular pathway (Pardridge, 1999). Microvasculature of central nervous system is consisted with non-fenestrated endothelium sealed by intercellular adherent junction and tight junction. Under normal functioning BBB, paracellular pathway is restricted by these structures.

As a part of CNS, retina also has functional barrier called blood-retinal barrier (BRB). Neural retina receives dual blood supply from retinal vessels and choroidal vessels. Retinal vessels and choroidal vessels are separated from neural retina by inner and outer BRB, respectively. In a narrow definition, inner BRB is a tight junction between retinal vascular endothelium (resembles the BBB proper of brain) and outer BRB means a tight junction between retinal pigment epithelium (resembles the blood-CSF barrier of brain). Among the two kinds of BRB, inner BRB is responsible for the pathogenesis of diabetic retinopathy.

Inner blood-retinal barrier is composed of diverse cellular component including endothelial cells, pericytes and Müller cells. Pericytes ensheathe the retinal microvascular endothelium and share their basement membrane with endothelial cells. Pericytes are connected to endothelial cells through the N-cadherin mediated adherent junction. Müller cells have

spatial proximity with endothelium and communicate with endothelium through their foot-processes. Each endothelial cell is interconnected with adjacent endothelial cell through tight junctions and adherent junctions to provide barrier function. Non-selective diffusion of molecules through the paracellular pathway is thightly regulated by those structures and limited exchange of molecules happens through the transcellular pathways (carrier mediated transport, transcytosis and lipophilic diffusion).
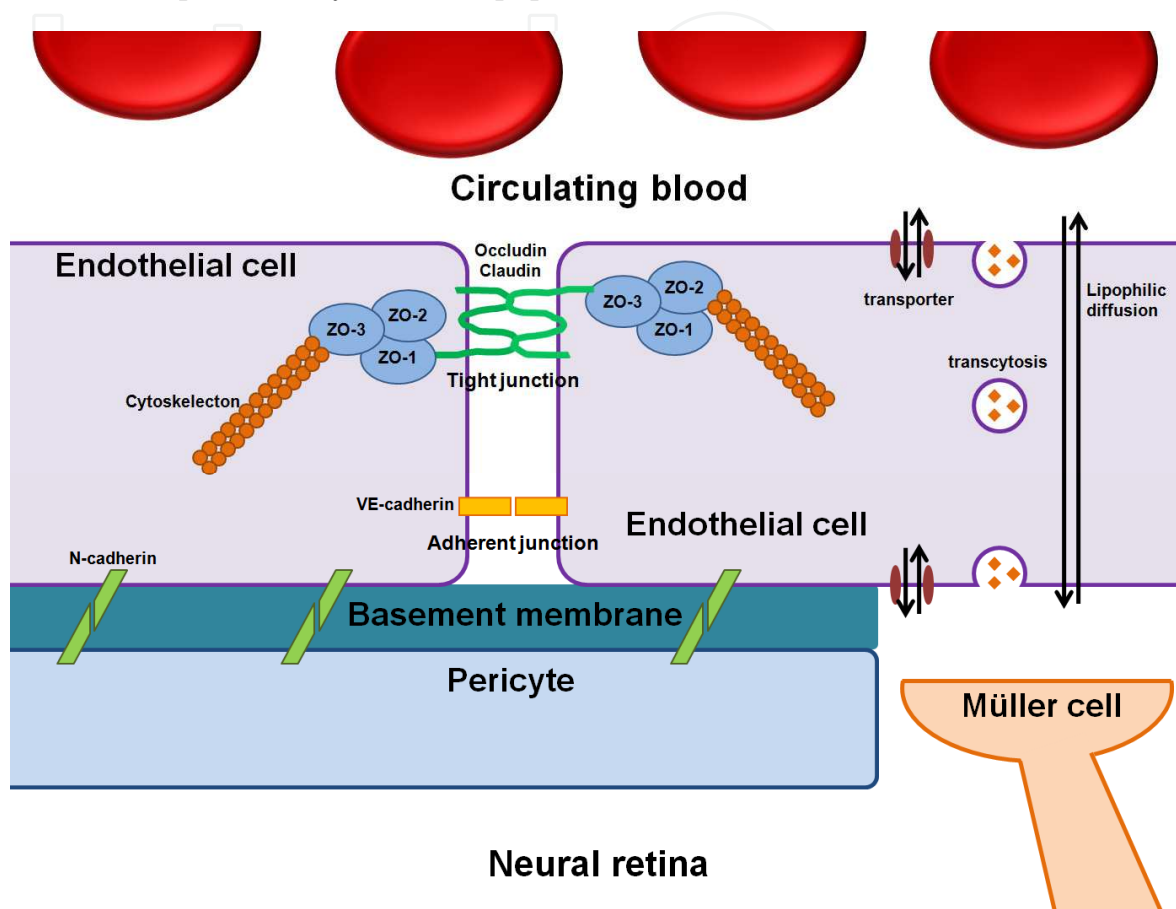

Fig. 1. A schematic view of the inner blood-retinal barrier

2.1 Molecular components of tight junction

Tight junction is constituted by various kinds of proteins. Transmembrane proteins like occludin and claudin interconnect endothelial cell with adjacent endothelium and exert a barrier function (Furuse et al., 1998; Russ et al., 1998). Cytoplasmic accessory proteins like zonular occludens (ZO) 1-3, cingulin, 7H6 antigen and cadherin-5 connect transmembrane proteins to cytoskelecton (Gumbiner et al., 1991; Persidsky et al., 2006). Tight junction proteins not only function as a barrier regulating paracellular diffusion, but also work as signaling complex (Gonzalez-Mariscal et al., 2008).

2.2 Molecular pathways across the BRB

Molecular exchange across the inner BRB is allowed through paracellular pathway and transcellular pathway (Pardridge, 1999). Paracellular pathway is restricted by tight junction and limited exchange of substances occurs according to a concentration gradient.

Transcellular pathway involves diffusion of lipophilic molecule, carrier mediated transport and transcytosis (Abbott et al., 2006).

2.3 Cellular components involved in the inner BRB

2.3.1 Pericyte

Retinal capillaries are covered by pericytes and pericytes exist in proximity to the endothelial cell (sharing basement membrane with endothelial cell). The spatial relationship facilitates the interaction of these two cells. Pericytes communicate with endothelial cells through diverse mediators like angiopoietins, transforming growth factor-β (TGF-β), platelet-derived growth factor-B (PDGF-B) and sphingosine-1-phosphate (S1P). Pericytes also form a heterocytic adherent junction with endothelial cells through N-cadherin (Navarro et al., 1998). In the retinal vasculature, the ratio of pericytes to endothelial cells is even higher than that of cerebral vasculature, reaching as high as 1:1.

Recently, pericyte has been spotlighted as a key player in the development and functional maintenance of blood-neural barrier. According to recent reports, the presence of pericyte is indispensable for the functional integration of endothelial cell and Müller cell. Moreover, its coverage of capillaries correlates with BBB integrity (Shepro & Morel, 1993). Several in vitro studies suggested that pericyte is an important cellular component in BBB regulation. Pericyte derived angiopoietin-1 induces occludin expression in brain capillary endothelial cell via Tie-2 receptor activation (Hori et al., 2004). TGF-β1/TGF-β receptor signaling between pericyte and endothelial cell plays an important role in enhancing BBB function (Dohgu et al., 2005). PDGF-B/PDGF receptor- β signaling is well known signal pathway involved in pericyte recruitment and proliferation during angiogenesis (Enge et al., 2002). For in vivo study, pericyte ablation model is needed, but Pdgfb -/- or Pdgfrb-/- mice, an ideal mural cell deficiency model, shows wide spread vascular leakage and hemorrhage leading to perinatal lethality (Leveen et al., 1994; Soriano, 1994). Perinatal lethality in these knock-out mice model made it difficult to analyze the role of pericyte in postnatal BBB dysfunction. More recently, studies using viable animal models of pericyte depletion provided an insight into the role of pericyte in BBB formation and regulation (Akagi et al., 1983; Thanabalasundaram et al., 2010).

The loss of pericyte is one of the earliest pathologic changes of diabetic retinopathy. The mechanism of pericyte loss in diabetic retinopathy is still unclear. Apoptosis triggered by hyperglycemia is presumed mechanism of pericyte loss in diabetic patients. Increased formation of advanced glycation end product (AGE) (Stitt et al., 1997) and aldose reductase expression in pericyte (Akagi et al., 1983) were suggested as the cause of pericyte loss under hyperglycemic condition. However, selective loss of pericyte is still to be elucidated because these mechanisms are common in various cell types. Pericyte loss not only results in the dysfunction of inner BRB, but also provides an important predisposing condition for the pathologic angiogenesis (Hammes et al., 2002). Under physiologic condition, pericytes inhibit the proliferation of endothelial cell. At the beginning of angiogenesis, the precedent denudation of pericytes from the pre-existing forefront of blood vessel is required for the mobilization of endothelial cells (Bergers & Benjamin, 2003; Yancopoulos et al., 2000). Endothelial hyperplasia which predispose for angiogenic sprouting can occur in the absence of pericytes.

2.3.2 Müller cells

In the development of primate retina, glial cells enter into the retina from the optic nerve and invade to the peripheral retina. Glial cells are involved in diverse process of retinal vascular development. First, glial cells secrete VEGF in response to hypoxic condition, resulting in retinal vascularization (Kim et al., 2010b). Second, a growing body of evidences showed that Müller cell, a predorminant constituent of the retinal glial cell, forms 'neurovascular unit' with endothelial cell and neuron to regulate blood flow of neural tissue and blood-neural barrier function. In vitro studies using retinal vascular endothelial cell co-cultured with glial cell or cultured with conditioned medium from glial cell demonstrated that glial cell is important in barrier properties including the expression of tight junction proteins (Gardner, 1995; Gardner et al., 1997). Several glial cell derived growth factors like angiopoietin-1, basic fibroblast growth factor (bFGF), glial derived growth factor (GDGF) and TGF-β are reported to induce the blood-neural barrier phenotype in vitro (Abbott et al., 2006). The src-suppressed C kinase substrate (SSeCKS) expressed in glial cell regulates the barrier integrity by the regulation of VEGF (potent mediator of vascular permeability) and angiopoietin-1 (involved in vascular maturation and barriergenesis) level (Lee et al., 2003).

Glial cell has a spatial proximity with endothelial cell and interconnected with basal laminar of endothelium via the end-foot processes. In the BBB, perivascular end-foot of glial cells contains abundant orthogonal arrays of particles (OAPs) in a polarized manner which is constituted with aquaporin 4 (AQP4) and the polarity of AQP4 localization in glial endfeet is disrupted under pathologic condition involving BBB impairment (Wolburg-Buchholz et al., 2009). In the perivascular endfeet of Müller cells, the expression of AQP4 also had been identified (Nagelhus et al., 1998). Agrin, an extracellular heparansulfate proteoglycan, is a factor that is known to affect this polarity of perivascular glial endfeet (Fallier-Becker et al., 2011; Wolburg et al., 2009) and suggested to be participated in the BBB development (Barber & Lieth, 1997).

Under diabetic condition both hypoxia and hyperglycemia can affect Müller cells leading to the breakdown of BRB. In the animal model of hypoxic retinopathy, hypoxia induced apoptotic loss of Müller cell leads to the subsequent BRB failure (Chan-Ling & Stone, 1992), pathologic angiogenesis and vitreous hemorrhage (Zhang & Stone, 1997). Moreover, in hypoxic retinopathy, Müller cell derived VEGF is essential pathogenic molecule resulting BRB disruption and pathologic neovascularization (Weidemann et al., 2010). Pathologic changes of retinal glia were also noted in the hyperglycemic condition. According to a study using streptozocin induced diabetic rat, Müller cell showed generalized regression throughout the retina from the early stage of diabetes before the BRB dysfunction (Rungger-Brandle et al., 2000). Furthermore, in diabetic rat, the alteration of perivascular Müller glial aquaporins was noted especially in the superficial retinal vessels which might affect the barrier function (Iandiev et al., 2007).

2.3.3 Endothelial cell

Retinal capillaries are consisted by non-fenestrated endothelium and the basement membrane of retinal vascular endothelium is continuous. Tight junction between retinal micro-vascular endothelial cells is the anatomical basis of the inner BRB. Retinal vascular endothelium forms a homocytic interconnection with adjacent cell via tight junction and adherent junction. Beside tight junction, adherent junction stabilizes the BRB providing mechanical force. VE-cadherin is major molecule involved in endothelial-endothelial

adherent junctional complex (Navarro et al., 1998). The luminal side of retinal vascular endothelium has negative charge due to the glycocalyx coat which contributes to the barrier function toward negatively charged molecules and the loss of this surface charge causes dysfunction of BRB (Lin, 1988).

Endothelial cell of retinal capillary expresses diverse transporters for selective molecular exchange between neural retina and circulation. Enzymatic activity vascular endothelial also contribute to barrier property through regulating the metabolism of substances from circulating blood to retina and vice versa.

2.3.4 Neuron

Retinal blood flow is tightly regulated according to the activity of retinal neurons. Metabolic need of ganglion cells is supposed to be an important factor in vascular development of retina. Intercellular communications between endothelial cells, Müller cells and neurons are expected to play a pivotal role in the formation and functioning of BRB. Diabetic retinopathy is a kind of progressive neuropathy. Retinal neuropathy in diabetes could be a consequence of preceding diabetic vascuopathy. However, there are some evidences that diabetes itself could be the cause of retinal neuropathy. In streptozocin induced early diabetic rats, increased apoptosis of neuron was documented especially in retinal ganglion cells and Müller cells (Hammes et al., 1995). Moreover, post-mortem human specimen from diabetic patients showed that apoptosis of neuronal cell could occur prior to clinically significant diabetic vasculopathy and the location of neuronal death had nothing to do with the presence of focal vascular lesions (Barber et al., 1998). These results suggest retinal neuronal apoptosis may not be the result of diabetic vasculopathy.

3. Disruption of inner blood-retinal barrier in diabetic retina

3.1 Protein Kinase C (PKC)

In the diabetic retina, cellular accumulation of diacylglycerol which activates PKC to translocate into plasma membrane and to acquire phosphorylation activities has been documented (Dempsey et al., 2000; Newton, 1997; Xia et al., 1994). Hyperglycemia induced the activation of PKC is associated with the pathologic changes of diabetic retinopathy. The exact mechanism of PKC induced vascular leakage in diabetic retinopathy still remains unclear, but PKC, especillay β-isoform is considered as a key mediator of VEGF induced BRB disruption and retinal neovascularization (Aiello et al., 1997; Xia et al., 1996). Recently, it is reported that PKCδ is also associated with the pathogenesis of diabetic retina through inducing the decrement of endothelial tight junction protein (ZO-1, 2) expression and subsequent vascular hyperpermeability in diabetic retina (Kim et al., 2010a). In addition, PKC mediated occludin phosphorylation is reported to participate in the VEGF stimulated vascular leakage (Harhaj et al., 2006). Some investigators also suggested nitric oxide (NO) pathway as a potential downstream target of PKC induced vascular permeability. In an experiment using coronary venule, PKC regulated vascular leakage partially relies on the endothelial NO synthesis (Huang & Yuan, 1997).

3.2 Advanced Glycation Endproducts (AGEs)

Long-term exposure to hyperglycemic environment results in a non-enzymatic glycation of protein, lipid and nucleic acid to form a heterogenous group of irreversible adducts called

AGEs. The clinical implication of AGEs is well documented in patients with diabetes. Among type 1 diabetic patients, skin levels of glycated collagen (Amadori product) and carboxymethyllysine (a kind of AGEs) showed correlation with the progression of diabetic retinopathy (Genuth et al., 2005). Vitreous level of hydroimidazolone, one of the most prominent AGEs, is reported to be increased in patients with type 2 diabetes (Fosmark et al., 2007).

AGEs and its receptor RAGE (receptor of AGEs) are known to exert a pivotal role in diabetic vascular complication such as retinopathy and nephropathy. Dysfunction of BRB in diabetic retinopathy is also caused by AGEs associated mechanism. First, dysfunction and apoptosis of pericyte, a key cellular component in the formation and maintenance of BRB are suggested as a mechanism of AGEs related BRB breakdown in diabetes. In streptozocin induced diabetic rats, significant deposition of AGEs and expression of RAGE were noted in pericytes of the capillary beds (Stitt et al., 1997). AGEs showed toxicity to pericyte in vitro and this toxic effect is mediated by AGE-RAGE interaction (Yamagishi et al., 1995). ROS generation through AGE-RAGE interaction results in oxidation of DNA, membrane lipid peroxidation and subsequent apoptotic pericyte death (Yamagishi et al., 2002b). In addition, AGEs regulate the expression of growth factors from pericyte which participate in the BRB function (Shimizu et al., 2011; Yamagishi et al., 2002a). Second, AGEs are involved in inflammatory reactions which cause the disturbance of BRB function. In diabetes, AGEs are accumulated in the vascular wall to stimulate proinflammatory reaction (Yan et al., 2003). These adducts not only activate leukocytes (Chibber et al., 2000) but also involved in the regulation of endothelial adhesion molecules. According to experimental study, blocking the interaction between AGEs and RAGE could effectively ameliorate retinal ICAM-1 expression, leukostasis and subsequent BRB breakdown in diabetic animal (Kaji et al., 2007).

Because diverse mechanisms are involved in the pathogensis of BRB breakdown in diabetic retinopathy, the evaluation on the effect of AGEs in non-diabetic indivisual is required for elucidating the role of AGEs in barrier dysfunction. In vivo studies using normo-glycemic animal showed that infusion of AGEs could induce vasculopathy resembling that of diabetes (Vlassara et al., 1992) and BRB breakdown associated with overexpression of VEGF (Stitt et al., 2000).

3.3 Sorbitol

Under hyperglycemia, glucose is converted to intracellular sorbitol by aldose reductase. Intracellular accumulation of sorbitol results in an osmotic damage to retinal vascular endothelium and pericytes. In a postmortem electron microscopic study of diabetic eye, BRB disruption and increased aldose reductase expression in the vascular cells participates in BRB formation (retinal vascular endothelial cells and Müller cells) were found which suggest that aldose reductase induced intracellular accumulation of sorbitol in vascular cell might contribute to the BRB breakdown in diabetes (Vinores et al., 1993). However, a randomized clinical trial of sorbinil, an aldose reductase inhibitor, in patients with diabetic retinopathy ended with little success in preventing retinopathy progression (Sorbinil Retinopathy Trial Research Group, 1990).

3.4 Vascular Endothelial Growth Factor (VEGF)

VEGF is not only a potent angiogenic growth factor, but also a strong vascular permeability enhancer. There are four different isoform of VEGF produced by alternative splicing of the

same gene: $VEGF_{121}$, $VEGF_{165}$, $VEGF_{189}$ and $VEGF_{206}$. Among these isoforms, $VEGF_{165}$ is the predominant isoform with optimal characteristics of bioavailability and bioactivity (Ferrara et al., 2003). The expression of VEGF is regulated mainly by oxygen tension. Under hypoxic condition, hypoxia-inducible factor (HIF)-1 binds to hypoxia response element (HRE) of the VEGF gene and activates genes participate in cellular response to hypoxia (Carmeliet et al., 1998; Jiang et al., 1996). VEGF expression is also controlled by diverse growth factors and inflammatory cytokines. In patients with proliferative diabetic retinopathy, vitreous level of VEGF is elevated and effectively reduced after panretinal laser photocoagulation (Aiello et al., 1994) because of a decreased metabolic need of neural retina which leads to amelioration of tissue hypoxia. Oxidative stress and pro-inflammatory cytokine is also suggested to be implicated in VEGF upregulation of diabetic retina (Frey & Antonetti, 2011; Giacco & Brownlee, 2010). VEGF exerts biological activity through specific receptor tyrosine kinases: VEGFR-1 and VEGFR-2. VEGFR-1 is considered as 'decoy' receptor which prevents VEGF from binding to VEGFR-2. Pro-angiogenic and vasopermeable effect of VEGF is mainly mediated by VEGF-2.

VEGF induces retinal vascular hyperpermeability through both transcellular pathway and paracellular pathway. VEGF induces pinocytotic vesicular transport by upregulation of the vesiculo-vacuolar organelle (VVO) formation (Feng et al., 1999). In paracellular pathway, VEGF affects both of tight junction and adherent junction. The expression and assembly of tight junction protein ZO-1 and occludin are reduced by the VEGF (Antonetti et al., 1998; Wang et al., 2001). Post-translational regulation of tight junction protein by phosphorylation is also responsible for the VEGF mediated vascular hyperpermeability (Antonetti et al., 1999; Harhaj et al., 2006). Phosphorylation and disorganization of VE-cadherin, a major component of adherent junction between microvascular endothelial cells are another pathogenic change associated with VEGF induced vascular leakage (Esser et al., 1998; Kevil et al., 1998). Moreover, in patients with diabetic retinopathy, over-expressed VEGF upregulates adhesion molecules (ICAM-1, VCAM-1 and E-selectin) and enhances leukocyte adhesion (Kim et al., 2001). During the process of angiogenesis, local concentration of VEGF also perturbs pericyte coverage and maturation of blood vessels (Greenberg et al., 2008). In the animal model of diabetes, the VEGF mediated BRB breakdown and the restoration of BRB by an inhibition of VEGF action are well documented from the early stage of diabetic retinopathy (Murata et al., 1995; Qaum et al., 2001). Now a days anti-VEGF agents are widely used in the treatment of diabetic macular edema.

3.5 Carbonic Anhydrase (CA)

CA is a ubiquitous enzyme that catalyzes the interconversion of carbon dioxide and bicarbonate to regulate pH of tissue and to help transport of carbon dioxide. Presence of CA in the posterior segment of human eye has been proven (Wistrand et al., 1986), but the significance of CA in the pathogenesis of diabetic retinopathy was underestimated until recently. According to comparative proteomic analysis of vitreous from non-diabetic, diabetic without retinopathy versus proliferative diabetic retinopathy (PDR) subjects revealed that vitreous concentration of CA-I in PDR group was 15.3 and 8.2 times higher than that of non-diabetic and diabetic without retinopathy groups, respectively. In the rat, intravitreal injection of CA caused retinal vascular hyperpermeability through different

mechanism form that of VEGF induced barrier breakdown. Authors postulated that in diabetic retinopathy, increased CA elevates intraocular pH which in turn activates kallikrein-kinin system and subsequent bradykinin receptor activation leads to BRB breakdown (Gao et al., 2007). Moreover, in streptozocin-induced diabetic rats, decreased kallikrein-binding protein level has been noted (Hatcher et al., 1997), which could increase the free kallikrein level. CA inhibitors are potential candidate of supplementary treatment option for diabetic macular edema. Actually in a pilot study with a few participants, acetazolamide, a CA inhibitor, showed partial effect in improving diabetic macular edema (Giusti et al., 2001).

3.6 Inflammation

It is now generally accepted that the pathogenesis of diabetic retinopathy involves low grade inflammation and vascular endothelial dysfunction (Gerhardinger et al., 2005; Joussen et al., 2004; van Hecke et al., 2005). Leukostasis of retinal microvasculature was consistently found from the early stage of diabetic retinopathy. Several experimental and clinical evidences indicated that leukostasis is one of the most important causative factors of typical diabetic microvascular pathologies such as microvascular acellularity, capillary drop-out and microaneurysm formation (Kim et al., 2005; Lutty et al., 1997; McLeod et al., 1995). Moreover, leukostasis in diabetic retinopathy is closely associated with BRB breakdown (Leal et al., 2007). In the diabetic retina, upregulation of VEGF and increased inducible NO synthase activity is involved in the expression of endothelial adhesion molecules like ICAM-1, VCAM-1 (Ishida et al., 2003; Leal et al., 2007; Nowak et al., 2008). Experimental study using ICAM knock-out mice revealed that adhesion molecule plays a key role in the endothelial dysfunction and barrier breakdown of diabetic retinopathy (Joussen et al., 2004).

Several inflammatory cytokines are participated in the breakdown of BRB in diabetes. Interleukin-1 (IL-1)β and tumor necrosis factor (TNF)-α are the representative inflammatory cytokines participate in the pathogenesis of diabetic retinopathy. Both in the vitreous humour and serum of patients with proliferative diabetic retinopathy, the level of IL-1β and TNF-α is increased (Demircan et al., 2006). The activity of caspase 1 which is a proteolytic enzyme involved in the production of IL-1β is also up-regulated in the retinas of diabetic patients (Mohr et al., 2002). IL-1β is a well known cytokine that induces barrier dysfunction through leukocyte recruitment in diverse pathologic condition. High concentration of glucose stimulates endothelial IL-1β over-expression and results in apoptosis of endothelial cell through the activation of NF-κB in vitro. Supplement of IL-1β caused retinal microvascular change resembling that of diabetic retinopathy (Kowluru & Odenbach, 2004) and inhibition of caspase-1/interleukin-1beta signaling with minocycline prevented vascular pathology of diabetic rat (Vincent & Mohr, 2007). TNF-α is also involved in the loss of retinal microvascular cells in diabetic retina (Behl et al., 2008). In bovine retinal endothelial cells, TNF-α disturbs the expression of tight junction proteins (claudin-5 and ZO-1) and subcellular localization of these proteins (Aveleira et al., 2010). In the TNF-α knock-out rat, diabetes associated retinal leukostasis, apoptosis of retinal microvascular cells and breakdown of BRB are significantly suppressed (Huang et al., 2011). Also in a diabetic rat, etanercept, a soluble tumor necrosis factor receptor (p75):Fc fusion protein (TNFR:Fc) effectively reduced leukostasis and breakdown of BRB (Joussen et al., 2002).

4. Clinical implication and current treatment modalities of diabetic inner BRB dysfunction: Diabetic macular edema

Macular edema which resulted from the dysfunctional BRB is the most common cause of visual disturbance in patients with nonproliferative diabetic retinopathy (NPDR). In addition to the disruption of inner BRB of the pre-existing retinal vasculature, the 'leaky' property of new vessels contributes to the macular edema in patients with proliferative diabetic retinopathy (PDR). Breakdown of BRB causes retention of fluid and plasma contents, such as lipoproteins within neural retina leading to retinal thickening. According to the data of Wisconsin epidemiologic study of diabetic retinopathy (WESDRP), the prevalence of diabetic macular edema ranges from 18 to 20% among the patients with diabetes (Klein et al., 1984a; Klein et al., 1984b).

After the reports of Early Treatment of Diabetic Retinopathy Study (ETDRS) focal/grid laser photocoagulation was the standard treatment method in diabetic macular edema. Stabilization or some improvement of vision was acquired in patients with macular edema who received laser photocoagulation. Although it is still the most cost-effective treatment modality in diabetic macular edema, some patients suffer from the post-treatment paracentral scotomas (Striph et al., 1988) and enlarging atrophic laser scars (Schatz et al., 1991). Although rare, vision threatening complications like choroidal neovascularization and subretinal fibrosis were also reported (Cunningham & Shons, 1979). Because of the refractory macular edema and complications of laser treatment, several pharmacological treatment modalities had been introduced. Further delineation on the exact mechanism of action is still needed, but intravitreal steroid injection is a powerful treatment option in diabetic macular edema. There are many in vitro and in vivo studies suggesting the mechanisms involved in the effect of corticosteroid treatment for diabetic macular edema. An in vitro study using bovine retinal endothelial cell monolayer showed that hydrocortisone treatment reduced monolayer permeability to water and solutes, increased tight junction proteins (ZO-1 and occludin) and reduced occludin phosphorylation (Antonetti et al., 2002). In experimental diabetic retina, corticosteroids demonstrated differential regulation of VEGF receptors (down-regulation of VEGFR-2 and up-regulation of VEGFR-1, a 'decoy' receptor) (Zhang et al., 2008), inhibitory effects on VEGF, ICAM-1 expression (Wang et al., 2008) and leukostasis (Tamura et al., 2005). Despite of the potential side effects like cataract and increased ocular pressure, intravitreal triamcinolone injection is one of the most commonly used treatment modality in diabetic retinopathy. More recently, several anti-VEGF agents are applicated in the treatment of diabetic macular edema. Several reports comparing the effectiveness of focal/grid laser photocoagulation, intravitreal triamcinolone injection and various anti-VEGF agents has been published (Diabetic Retinopathy Clinical Research Network, 2008; Soheilian et al., 2009), but more large scale studies with prolonged observation period are needed.

5. Possible therapeutic approach to diabetic retinopathy through BRB modulation

In addition to previously commented treatment modalities, diverse therapeutic agents had been suggested for the medical treatment of diabetic retinopathy through the modulation of inner BRB disruption. Clinical studies involving medical treatment of inner BRB dysfunction are summarized in table 1 and each therapeutic candidate is further delineated below.

Drug (Class)	Suggested mechanism	Clinical trials		
		Study	Population (N:enrolled/completed)	Result
Fenofibrate (Fibrate)	Inhibit VEGF production Reduce adhesion molecule level	FIELD study	Type 2 DM patients without requiring lipid modifying treatment (9795/9764)	Reduced the risk of ME which needs laser treatment
Lisinopril (ACE inhibitor)	Inhibit VEGF production	EUCLID	Non-hypertensive type 1 DM patients (530/354)	No significant effect on the retinopathy progression
Candesartan (AT1R blocker)	Inhibit VEGF production	DIRECT-prevent 1	Normoalbuminuric, normotensive type 1 DM patients (1421/1421)	Reduce the risk of retinopathy
		DIRECT-protect 1	Normoalbuminuric, normotensive type 1 DM patients (1905/1905)	No significant preventing effect on both retinopathy progression and ME development
		DIRECT-protect 2	Normoalbuminuric, type 2 DM (1905/1905)	
Telmisartan (AT1R blocker)	Reduce RAGE expression	Untitled (Nakamura et al., 2005)	Patients with essential hypertension (7/7)	Decrease serum levels of sRAGE
Ruboxistaurin (PKC inhibitor)	Blocking VEGF mediated tight junction dysregulation	PKC-DRS	Patients with moderate to severe NPDR (252/157)	No significant effect on both retinopathy progression and ME development Reduced the risk of MVL
		PKC-DRS2	Patients with moderate to severe NPDR (685/514)	Reduced the progression of ME into the center of macula and need for laser treatment for ME
		PKC-DMES	Patients with DME farther than 300 μm from central macula, an ETDRS retinopathy level from 20 to 47A (686/506)	Preventive effect on ME progression
Pimagedine (Aminoguanidine)	Lowering AGEs production	ACTION I trial	Patients with type 1 DM (690/472)	Significantly reduced the risk of three-step or greater progression of the retinopathy score

Abbreviations
ACTION I: A Clinical Trial In Overt Nephropathy of Type 1 Diabetics, AT1R: angiotensin II type 1 receptor, DIRECT: Diabetic Retinopathy Cardesartan Trials, ETDRS: Early Treatment of Diabetic Retinopathy Study, DM: diabetes mellitus, EUCLID: EURODIAB Controlled Trial of Lisinopril in Insulin-Dependent Diabetes Mellitus, FIELD: Fenofibrate Intervention and Event Lowering in Diabetes, FU: follow-up, ME: macular edema, MVL: moderate visual loss, PKC-DRS: PKC inhibitor diabetic retinopathy study, PKC-DMES: PKC inhibitor diabetic macular edema study, sRAGE: soluble form of RAGE.

Table 1. Possible medical therapeutic agent for the treatment of diabetic retinopathy through a modulation of inner BRB disruption

5.1 Fenofibrate

Because dyslipidemia is a well documented risk factor of diabetic macular edema and hard exudates deposition, lipid lowering treatment was expected to have benefit on these complications. The Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study group applicated fenofibrate, a peroxisome proliferator-activated receptor (PPAR)-α agonist which is widely used as lipid lowering agent in diabetic patients to reduce the risk of microvascular and macrovascular complications. PPAR-α agonist not only modulates lipid composition, but also inhibits the production of VEGF (Panigrahy et al., 2008) and reduces serum levels of adhesion molecule VCAM-1 and ICAM-1 (Rosenson et al., 2007) which are key components in the pathogenesis of BRB breakdown. Fenofibrate treatment demonstrated significant preventive effect on the hypoxia induced endothelial hyperpermeability of an in vitro BBB model (Mysiorek et al., 2009). In the type 2 diabetes patients without requiring lipid-modifying treatment, mean 5 years of fenofibrate (200 mg/day) treatment significantly reduced the risk of macular edema development which needs laser treatment (31% reduction) (Keech et al., 2007).

5.2 Blocking of the Retina-Angiotensin System (RAS)

In human eye, the local expression of RAS components: renin, angiotensin converting enzyme (ACE) and angiotensin has been reported (Wagner et al., 1996) and their activation in diabetic retinopathy (Danser et al., 1989) is well documented. Increasing evidences indicate that angiotensin II stimulates the expression of VEGF in vitro (Williams et al., 1995). In streptozotocin-induced diabetic animal, ACE inhibitor treatment inhibited retinal VEGF production and subsequent retinal vascular hyperpermeability associated with BRB breakdown (Kim et al., 2009). Moreover, among the patients with proliferative diabetic retinopathy, vitreous level of VEGF is significantly lower in patients receiving ACE-inhibition (Hogeboom van Buggenum et al., 2002). In diabetic hypertensive rat, treatment with candesartan, an angiotensin II receptor blocker effectively suppressed the vascular permeability across the BBB (Awad, 2006).

On these experimental bases, several RAS inhibiting agents had been applied for the treatment of diabetic retinopathy. The EURODIAB Controlled Trial of Lisinopril in Insulin-Dependent Diabetes Mellitus (EUCLID) suggested that lisinopril, an ACE inhibitor could have benefit on the progression of diabetic retinopathy in patients with type 1 diabetes. However, the primary endpoint was the progression of diabetic retinopathy, and the protective effect does not showed statistical strength (Chaturvedi et al., 1998).

The Diabetic Retinopathy Cardesartan Trials (DIRECT) group has performed three separate randomized, double-blind, placebo-controlled clinical trials to evaluate the efficacy of candesartan on reducing the incidence (DIRECT-Prevent 1), progression of retinopathy in type 1 (DIRECT-Protect 1) (Chaturvedi et al., 2008) and the progression of retinopathy in type 2 diabetes patients (DIRECT-Protect 2) (Sjolie et al., 2008). In both DIRECT-Protect 1 and 2, the primary endpoint was progression of diabetic retinopathy, which was defined as three or more step deterioration of ETDRS level. Development of clinically significant macular edema (CSME), development of proliferative diabetic retinopathy or both were settled as secondary endpoint. Five years of candesartan treatment did showed significantly increased probability of diabetic retinopathy regression (34% increment) in type 2 diabetes patients, but there were no significant preventing effects on the progression of disease in

both type 1 and 2 patients. Although it was not the primary endpoint, the incidence of CSME development was not affected by candesartan treatment.

5.3 Protein kinase C inhibitors

Since Ishii et al. (Ishii et al., 1996) demonstrated that LY333531, a selective inhibitor of PKC β-isoform could rescue diabetic animals from vascular dysfunction, several clinical studies on ruboxistaurin (orally active form of selective PKC β inhibitor: LY333531) use for diabetic retinopathy had been performed: the PKC inhibitor diabetic retinopathy study (PKC-DRS), the PKC inhibitor diabetic retinopathy study 2 (PKC-DRS2), the PKC inhibitor diabetic macular edema study (PKC-DMES). PKC-DRS is a phase 3, multicenter, double-masked, placebo controlled trial involving patients with moderate to severe NPDR, the endpoints of which are progression of diabetic retinopathy (equal to or greater than three-step worsening in the ETDRS scale), moderate vision loss (MVL: vision decrease of three or more lines on the ETDRS chart) and sustained MVL (SMVL: MVL in two consecutive visit 6 or more months apart). Oral administration of ruboxistaurin (32 mg/day) for more than 36 months showed no preventive effect on the diabetic retinopathy progression, but significantly delayed the occurrence of MVL and SMVL and reduced the risk of MVL to one-third of that in the placebo group (The Protein Kinase C beta Inhibitor Diabetic Retinopathy Study [PKC-DRS], 2005). According to PKC-DRS2, a following phase 3 clinical trial designed to evaluate visual outcome of ruboxistaurin treatment in patients with moderate to severe NPDR, administration of ruboxistaurin reduced a 3-year risk of SMVL from 9.1% to 5.5% (40% risk reduction). Moreover, ruboxistaurin reduced the progression of macular edema into the center of macula and need for laser treatment for macular edema (Aiello et al., 2006). PKC-DMES, an multicenter, double-masked, randomized placebo controlled trial the endpoint of which was progression to sight threatening macular edema or application of photocoagulation for diabetic macular edema, revealed partial effect of ruboxistaurin on the progression of diabetic macular edema to a more severe form in patients with diabetic macular edema (The Protein Kinase C beta inhibitor diabetic macular edema study [PKC-DMES], 2007).

5.4 Blockade of AGE-RAGE pathway

5.4.1 Lowering AGEs production

Aminoguanidine is a prototype drug for the prevention of diabetes-induced AGEs formation in vivo (Brownlee et al., 1986). The pharmacological mechanism of aminoguanidine involves inhibition of NO synthases and semicarbazide-sensitive amine oxidase. Hammes et al. insisted aminoguanidine treatment effectively inhibited the retinal arteriolar accumulation of AGEs, prevented abnormal endothelial proliferation and pericyte loss in diabetic rat. Acellular capillaries and microaneurysms, typical pathologic findings of diabetic retinopathy were also reduced significantly in the aminoguanidine treatment group (Hammes et al., 1991). Administration of aminoguanidine effectively attenuated cellular loss and microthrombus formation of retinal vessels also in diabetic spontaneous hypertensive rats (Hammes et al., 1994). In these reports, authors adapted in situ detection of advanced glycosylation-specific fluorescence for the quantification of AGEs, which might not be specific for AGEs, but also detect other oxidation products. Kern et al. found that aminoguanidine treatment effectively reduces pericyte loss, formation of microaneurysms

and acellular capillaries, but inhibitory effect of retinal AGEs accumulation is not significant in diabetic dogs (Kern & Engerman, 2001). A randomized, placebo-controlled study in patients with type 1 diabetes mellitus showed that treatment with pimagedine, a kind of aminoguanidine significantly reduced the risk of three-step or greater progression of the retinopathy (ETDRS) score (Bolton et al., 2004). The exact pharmachologic mechanism of aminoguanidine in preventing diabetic retinopathy progression is still to be elucidated and more clinical trials are needed to clearly delineate the benefit of aminoguanidine treatment in diabetic retinopathy.

5.4.2 Lowering RAGE expression

Several commonly used therapeutic agents showed effects on the reduction of RAGE expression in vascular endothelial cell: thiazolidinediones (rosiglitazone and pioglitazone), calcium channel blocker (nifedipine), angiotensin II receptor blocker (telmisartan). Rosiglitazone and pioglitazone, kinds of thiazolidinediones, an anti-diabetic drug act by binding to peroxisome proliferator-activated receptors reduces basal and tumor necrosis factor-α stimulated expression of RAGE in cultured human umbilical vein endothelial cells. Decreased RAGE by thiazolidinediones results in subsequent inhibition of AGEs stimulated expression of pro-inflammatory protein (Marx et al., 2004). Nifedipine, a calcium channel blocker inhibits RAGE upregulation in AGEs treated human umbilical vein endothelial cells to reduce AGEs induced ROS production (Yamagishi & Takeuchi, 2004). Telmisartan, an angiotensin II receptor blocker inhibits RAGE expression in cultured human microvascular endothelial cells in vitro and decreases serum soluble form of RAGE level in patients with essential hypertension (Nakamura et al., 2005). In vivo studies evaluating the effect of those agents on the inner BRB breakdown in diabetic retinopathy are needed.

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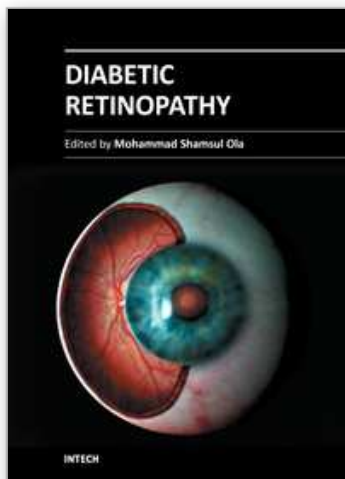
Diabetic Retinopathy

Edited by Dr. Mohammad Shamsul Ola

The aim of this book is to provide a comprehensive overview of current concepts in pathogenesis, diagnosis and treatments of diabetic retinopathy. It provides a collection of topics written by excellent authors, covering discussions on advances in understanding of pathophysiology, immunological factors and emerging concepts, relating to clinical aspects and treatment strategies. The contents of the book will not only provide a resource for our knowledge but also improve diagnosis and treatment options for those patients who suffer vision loss due to diabetic retinopathy.

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