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Blood Brain Barrier Injury in Diabetes: Unrecognized Effects on Brain and Cognition

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Abstract

Diabetes mellitus (DM) is a disorder due to the inability properly to metabolize glucose associated with dysregulation of metabolic pathways of lipids and proteins resulting in structural and functional changes of various organ systems. DM has detrimental effects on the vasculature, resulting in the development of various cardiovascular diseases and stemming from microvascular injury. The blood brain barrier (BBB) is a highly specialized structure protecting the unique microenvironment of the brain. Endothelial cells, connected by junctional complexes and expressing numerous transporters, constitute the main cell type in the BBB. Other components, including pericytes, basement membrane, astrocytes and perivascular macrophages, join endothelial cells to form the neurovascular unit (NVU) and contribute to the proper function and integrity of the BBB. The role of the BBB in the pathogenesis of diabetic encephalopathy and other diabetes-related complications in the central nervous system is apparent. However, the mechanisms, timing and consequences of BBB injury in diabetes are not well understood. The importance of further studies related to barrier dysfunction in diabetes is dictated by its potential involvement in the cognitive demise associated with DM. This review summarizes the impact of DM on BBB/NVU integrity and function leading to neurological and cognitive complications.

Keywords

diabetes; blood brain barrier; endothelial cell; pericyte; cognitive impairment; neuroinflammation

Introduction

Neurocognitive and neurologic complications including vascular dementia, stroke, anxiety/depression and cognitive impairment are well documented in diabetes mellitus (DM) (Prasad et al., 2014). Furthermore, DM can aggravate epilepsy (Schober et al., 2012) (McCorry et al., 2006) and stroke (Peng et al., 2013), leading to increased mortality. Diabetic encephalopathy, an important complication of diabetes, increases the probability of cognitive decline, acceleration of Alzheimer's disease and other forms of dementia (Summers, 2004;

Pasquier et al., 2006). Such clinical outcomes in DM are multifactorial and are related to both macrovascular changes and microvascular injury due endothelial dysfunction leading to end-organ pathology (Alexandru et al., 2016). Substantial evidence from recent clinical and experimental studies suggests that prolonged hyperglycemic conditions, particularly in DM type 2, lead to the progressive impairment of neuronal function in the brain (Mooradian, 1997). Longstanding DM type 2 is also associated with the disruption of neurovascular coupling associated with vascular dementia (Mogi and Horiuchi, 2011). Neuroimaging studies revealed smaller brain volumes in patients with DM and white matter lesions thought to be associated with blood brain barrier (BBB) injury (Mogi and Horiuchi, 2011). Impairment of the BBB/neurovascular unit (NVU) is now considered to be one of the critical factors leading to development of diabetic encephalopathy (Norhammar et al., 2002; Allen and Bayraktutan, 2009). The role of BBB dysfunction in the pathogenesis of diverse neurologic conditions is unquestionable; however, it has not been well-studied in DM (Prasad et al., 2014) as compared to diabetic retinopathy or renal complications.

Structural adaptation of the BBB in DM

Compromise of the structural integrity and transport function of brain endothelium has been implicated in BBB injury associated with the hyperglycemia, oxidative stress, and chronic inflammation seen in DM. The BBB is a specialized structural barrier, which regulates exchange between the blood and brain microenvironment, thereby maintaining homeostasis. Located at the interface between blood and brain parenchyma, the BBB is composed of a tight monolayer of brain microvascular endothelial cells (BMVECs), pericytes, basement membrane, and astrocytic end feet. Other components of the NVU are perivascular macrophages and neurons (Persidsky et al., 2006a; Abbott et al., 2010) (Figure 1, panel 1). Barrier permeability is an active and dynamic process based on the size and biochemical properties of the substrate and physiologic state of BBB elements (Persidsky et al., 2006a; Abbott et al., 2010).

The BMVEC monolayer is connected by junctional complexes consisting of adherent, gap, and tight junctions (Abbott, 2000; Persidsky et al., 2006a). Tight junctions (TJ) are considered the main structures responsible for the establishment of the BBB. Integrity of the TJ assembly is a vital factor to the very low paracellular permeability of hydrophilic molecules (Persidsky et al., 2006a). TJs are comprised of integral transmembrane proteins including occludin, claudins, junctional adhesion molecules, and endothelial cell-selective adhesion molecules (Suzuki et al., 2002; Persidsky et al., 2006a; Wegmann et al., 2006). Trans-membrane TJ proteins are connected to the actin-based cytoskeleton by association with ZO-1, ZO-2, ZO-3, cingulin, AF6, and 7H6, which also may have a role in regulating TJ tightness (Persidsky et al., 2006a; Fanning et al., 2007; Chen et al., 2015).

In vitro and *in vivo* experimental models have reported compromised integrity of the BBB under diabetic conditions (Pasquier et al., 2006; Wang et al., 2012b). Morphologic modifications of brain micro- and macro-vessels include thickening of the basal lamina with collagen deposition, accumulation of lipid peroxidation byproducts, and degeneration of pericytes and endothelial cells (McCuskey and McCuskey, 1984; Junker et al., 1985; Bouchard et al., 2002). Diabetes can also result in anomalous cerebral neovascularization

and increased blood capillary density within the central nervous system (CNS) (Figure 1, panel 2). This remodeling serves to extend vascular damage and risk of leakage associated with neurodegenerative processes (Prakash et al., 2013). It has been shown that enhanced BBB permeability and DM correlated with development of dementia while amyloid deposition or APOE genotype (major predictors of Alzheimer's disease) had no impact (Janelidze et al., 2017). Importantly, increased permeability positively correlated with cerebrospinal fluid (CSF) biomarkers of angiogenesis and endothelial dysfunction [vascular endothelial growth factor (VEGF), the ratio of VEGF/ soluble VEGF receptor (sVEGFR-1), ICAM-1, and VCAM-1). VEGF or the VEGF/sVEGFR-1 ratio was increased in all dementias and in minor cognitive impairment, whereas patients with diabetes showed increased CSF levels of VEGF, ICAM-1, and VCAM-1.

Increased permeability of BBB was detected in aging rhesus monkeys with DM type 2 by dynamic contrast-enhanced magnetic resonance imaging (Xu et al., 2016). Immunohistochemistry showed decrease in ZO-1 staining in cerebral microvessels and perivascular IgG leaked out of the blood. Enhanced permeability in DM has been demonstrated by IgG staining (Acharya et al., 2013) and [¹⁴C]sucrose accumulation (Hawkins et al., 2007) in animal models and by gadolinium tracer accumulation by magnetic resonance in DM type 2 patients (Starr et al., 2003). Not all regions of the BBB are equally susceptible to injury in a rat model of diabetes as demonstrated by Huber et al. (Huber et al., 2006). The midbrain is the first to show enhanced leakage after 28 days of diabetes, followed by hippocampus, cortex, and basal ganglia. Other regions showed no disruption even after 3 months.

Astrocytes, pericytes, and perivascular macrophages modulate BBB function (Persidsky et al., 2006a). Pericytes are enveloped within the basal lamina and provide direct structural support to the cerebral endothelium as well as metabolic support by the release of growth factors (Winkler et al., 2011). Perivascular macrophages are located abluminally within the basal lamina and serve as phagocytes in the perivascular environment. Interactions between activated brain macrophages and astrocytes can increase inflammatory responses and chemokine production leading to BBB disruption and enhanced leukocyte migration (Persidsky, 1999; Persidsky et al., 1999; Stamatovic et al., 2005). Activated perivascular astrocytes show augmented expression of glial fibrillary acidic protein (GFAP), which might be a marker of BBB dysfunction and injury (Mooradian, 1997; Mogi and Horiuchi, 2011).

Metabolic pathway disturbance leads to BBB dysfunction

The initiation of vascular complications in DM is linked to mitochondrial superoxide overproduction in the endothelium. Hyperglycemia within insulin-independent cells drives an accelerated rate of glucose metabolism, leading to excessive generation of reactive oxygen species (ROS) by the electron transport chain. The accumulation of ROS has a variety of deleterious effects on cellular functions. Using co-culture of human BMVEC and astrocytes, reversible decrease of TJ integrity and trans-endothelial electrical resistance (TEER) in high glucose (HG) conditions (25 mM D-glucose) was observed after five days of exposure (Mogi and Horiuchi, 2011). Co-treatment with antioxidant compounds, free radical scavengers, and antioxidant enzymes attenuated the observed effect. To further test the

hypothesis that oxidative stress in diabetes is caused by the production of ROS during respiration, the effect of HG on respiration rate and ROS production were investigated in mouse brain pericytes (Shah et al., 2013). The rate of respiration and ROS production were shown to be elevated in cultured brain pericytes in response to HG. However, when the activity of mitochondrial carbonic anhydrases was inhibited, a decrease in rate of respiration and ROS production was observed. These data provide new evidence for pharmacologic inhibition of mitochondrial carbonic anhydrases as protective against hyperglycemia-induced oxidative stress in the brain (Min et al., 2012).

BBB dysfunction can be further promoted by impairment of glycolysis by ROS and subsequent buildup of glycolytic intermediates, which distribute into four distinct metabolic pathways: polyol, hexosamine biosynthesis, protein kinase C (PKC), and advanced glycation end product (AGE) pathways (Brownlee, 2001; Dias and Griffiths, 2014).

While the contribution of these metabolic pathways to BBB pathology is not fully understood, substantial *in vitro* evidence suggests a role for PKC. The previously mentioned study utilizing co-culture of human BMVECs and astrocytes (Mogi and Horiuchi, 2011) noted that inhibition of PKC completely attenuated the increased permeability caused by HG. A similar study with triple culture (BMVECs, astrocytes and pericytes) replicated these findings and further showed that PKC activation leads to increases of NADPH oxidase and matrix metalloproteinase 2 (MMP-2), while decreasing occludin expression (Shao and Bayraktutan, 2013). Isoforms PKC- β and PKC- β II had the greatest impact.

AGEs and their receptor (RAGE) have a pivotal role in the potentiation of vascular pathology, even after glycemic control is achieved. Accelerated formation of AGEs results from months to years of hyperglycemia and persists as adducts and cross-linking agents for a wide variety of protein species (Ahmed, 2005; Ceriello, 2012). Aside from the consequences of widespread protein modification, AGEs act through RAGE to further amplify cellular dysfunction driven by oxidative stress and chronic inflammatory responses (Serban et al., 2016). RAGE activates NF- κ B and p21ras (Mooradian, 1997), inducing vascular inflammation (Mogi and Horiuchi, 2011), thrombosis, and enhanced expression of RAGE. This cycle promotes oxidative stress via generation of ROS by inflammatory signaling, leading to an imbalance with cellular antioxidant defense and tissue injury (Mogi and Horiuchi, 2011; Wang et al., 2012b). Additionally, the overproduction of ROS results in activation of endothelial adhesion molecules, such as VCAM-1, to further increase vascular permeability and monocyte adhesion (Kim et al., 2015; Patrick et al., 2015). Decreased expression of eNOS and prostacyclin leads to the impairment of endothelium-dependent vasodilation (Mooradian, 1997; Brownlee, 2001; Safiah Mokhtar et al., 2013). These phenomena have been named 'metabolic memory' and correlate with clinical observations regarding the importance of early glycemic control on long-term outcomes in patients with DM (Ceriello, 2012).

Damage to vessels as the result of cycles of inflammation and generation of ROS is a point of convergence in DM pathology; however, acute hyperglycemia versus the persistence of AGEs arrives at this outcome by distinct mechanistic routes. The distinct effects of each have been examined experimentally, either through *in vitro* exposure to HG versus AGEs or

with *in vivo* comparison of controlled versus untreated diabetes. The resultant pathological changes are frequently indistinguishable and the activities of each pathway are not mutually exclusive in disease; however, there has been an attempt to differentiate the unique effects of each as described in subsequent sections of this review.

Endothelial cell dysfunction

The initial gross morphological changes in diabetic encephalopathy include down regulation of TJ proteins, resulting in decreased tightness and greater size-dependent permeability of the BBB (Huber et al., 2006). In a Type 2 DM mouse model, these changes have coincided with cognitive impairment as assessed by the Morris Water Maze. Additionally, there is an observable increase in monocyte chemoattractant protein 1 (MCP-1)/CCL2, tumor necrosis factor α (TNF α), interleukin 6 (IL-6), and superoxide ion in brain tissue isolates of these same animals, consistent with other forms of inflammation involving leukocyte-brain endothelium engagement (Serban et al., 2016). Angiotensin II type 1 receptor blockade and peroxisome proliferator-activated receptor- γ activation attenuate all the effects noted.

In Type 1 DM patients with diabetic ketoacidosis (DKA), brain edema is a frequent occurrence and results from neuroinflammation and TJ compromise (Zhong et al., 2012). DKA is associated with high levels of serum IL-6, and an abundance of CCL2, NF- κ B, and nitrotyrosine found in brain tissue by neuropathologic examination (Yorulmaz et al., 2015). Brain tissue is characterized by the absence of TJ proteins including ZO-1, occludin, JAM-1, and claudin-5, paralleling edema. Chronic DM type 2 led to increased permeability of BBB and diminished expression of TJs (occludin, claudin-5) in hippocampus in rats (Yoo et al., 2016a). It has been shown recently that hippocampal BBB permeability in rats increased when these animals were fed a high fat/high sugar diet provided that they gained weight vs. ones did not gain weight (Davidson et al., 2012). Further, the enhanced permeability was associated with memory impairments (particularly hippocampus dependent learning). These observations together provide links between cognitive impairment, BBB dysfunction and obesity.

Results obtained with *in vitro* BBB models exposed to HG showed effects similar to observations in human and animal studies. HG conditions caused a decrease of BBB integrity and partial loss of ZO-1, occludin, and claudin-5 in co-culture of human BMVECs with astrocytes. NADPH oxidase activity (Mogi and Horiuchi, 2011) and the generation of superoxide anions were enhanced proportionally to increased BBB permeability (Ahmed, 2005). These changes occurred with exposure to 25 mM D-glucose after five days and were not observed with normoglycemic osmotic insult. Inhibition of p38 MAPK reversed BBB permeability; the activation of AMP-activated protein kinase was able to prevent induction of NADPH oxidase-derived superoxide anions and TJ protein degradation driven by HG.

Angiogenic signaling by VEGF appears to mediate the effects of HG. Transcription and expression of the upstream transcription factor, hypoxia inducible factor 1 (HIF-1), is elevated by HG in mouse BMVECs and associated with down regulation of occludin and ZO-1 (Chronopoulos et al., 2010; Tien et al., 2014). A blockade of VEGF in these cells ameliorated the downstream impact on TJs, suggesting that VEGF is produced by BMVECs

in response to HIF-1 and acts in an autocrine/paracrine fashion to increase BBB permeability (Figure 2). Further evidence from more generalized forms of inflammation demonstrates that astrocytes are also able to modulate BMVECs via paracrine release of VEGF (Tien et al., 2014).

Levels of serum VEGF differ substantially from local tissue expression at the BBB and may also play a significant role in transport of glucose under critical conditions. In studies examining circulating human VEGF, there is a divergence from the local BMVEC effects previously mentioned, with hyperglycemia decreasing and hypoglycemia increasing plasma VEGF levels (Oltmanns et al., 2008). Increased VEGF results in greater glucose transport across the BBB and may act as a neuro-protective agent for constant cerebral glucose supply (Oltmanns et al., 2008; Bates, 2010). These findings implicate systemic VEGF as a vascular permeability factor independent of local VEGF; both are likely to play a part in hyperglycemia and hypoglycemia-induced BBB dysfunction.

The impact of *in vitro* AGE treatment on BBB models shows consistent results; however, associated signaling events have yet to be elucidated. One study used co-culture of immortalized human BMVECs, astrocytes, and pericytes grown to mimic the properties of BBB (Wang et al., 2012a). Interestingly, AGEs treatment induced a downregulation of claudin-5, while leaving expression of occludin and ZO-1 unchanged. This effect was RAGE-dependent, accompanied by increased permeability, and required the same autocrine action of VEGF as seen HG. Changes in TJ after exposure to AGEs is mediated by matrix MMPs (Hawkins et al., 2007; Zhong et al., 2012; Shimizu et al., 2013; Persidsky et al., 2016). A drop in occludin and ZO-1 expression was associated with increasing MMPs in rat and human DM (Zhong et al., 2012; Shah et al., 2013), and inhibition of MMP-9 prevented increased permeability in streptozotocin (STZ)-induced DM in mice (Shimizu et al., 2013). The AGEs-mediated decrease in claudin-5 *in vitro* can be abolished through blockade of MMPs (Wang et al., 2012a), further suggesting that MMPs are necessary for AGEs-mediated damage (Figure 2). Lastly, it is possible that some part of the AGE-mediated effects result from the action of AGE on pericytes, leading to indirect modulation of BMVEC through TGF- β . *In vitro*, a change in claudin-5 expression and TEER can be prevented by pre-treatment with TGF- β antibodies (Wang et al., 2012a) (Figure 2). Pericytes are a known source of TGF- β in DM. Although the effect of AGEs on BMVECs appears to be RAGE-dependent, there have not been any studies examining RAGE action in isolated BMVECs.

Several studies examining TJ expression profiles after AGEs treatment suggest that the specifics are highly dependent upon the experimental model used. Occludin expression was decreased in two rat models of DM (Persidsky et al., 2016; Yoo et al., 2016b). A human monoculture study (Argaw et al., 2009) replicated this finding, while also observing decreased claudin-5 and no effect on ZO-1. Although further studies are needed, results obtained in several models suggest that ZO-1 modulation is unique to HG treatment and unchanged with exposure exclusively to AGEs.

The paracellular route of entry is of primary importance when discussing BBB dysfunction; however, evidence suggests that transcellular mechanisms of efflux may be impaired as well. P-glycoprotein (P-gp) is an efflux pump with broad specificity and it plays a significant role

in protecting the CNS from the penetration of exogenous toxins. Primary expression of P-gp is in the luminal membrane of BMVECs and in choroid plexus epithelium (Kwan and Brodie, 2005). Diminished P-gp function results in greater CNS vulnerability to circulating toxins. Its expression in DM is inconclusive, as mouse models have shown decreases, increases, and lack of change depending on the study (Liu et al., 2006; Dinapoli et al., 2010; Reichel et al., 2011). In STZ-induced diabetes, P-gp level was normalized after therapy with insulin (Dave et al.).

Astrocyte dysfunction

Like other components of the BBB, exposure of astrocytes to the longstanding effects of DM results in cell-specific dysfunction. In diabetic mice, electron microscopy revealed swollen astrocytic end feet in small vessels as well as detachment of the plasma membrane from basal lamina (Min et al., 2012). These observations correlated with increased functional measurements of permeability. Prolonged hyperglycemia may also have a negative impact on astrocytic regulation of blood flow (Alvarez et al., 2013).

Reduction of communication between astrocytes via gap junctions has been shown in cultured cells exposed to HG and brain slices obtained from diabetic rats (Gandhi et al., 2010). The treatment of human astrocytes with 15 mM glucose improved cell viability *in vitro*, while 30 mM glucose had a detrimental effect on astrocytes, resulting in morphological alterations along with changes in the expression of cytoskeletal proteins, including GFAP and vimentin (Wang et al., 2012a). Levels of TNF α , IL-6, IL-1, IL-4 and VEGF were also increased, while TGF- β levels remained unchanged. Similar to other cell types, HG-triggered insult was mediated via overproduction of ROS and activation of NF- κ B and STAT3 inflammatory pathways. Treatment with ROS scavengers and NF- κ B and STAT3 specific inhibitors suppressed HG-induced overexpression of inflammatory factors and VEGF (Wang et al., 2012a).

Pericyte dysfunction

Pericytes located in close apposition to the abluminal cell membrane of BMVEC are significantly affected in DM. Being in close proximity to BMVEC, they contribute substantially to BBB integrity; however, these cells are highly susceptible to metabolic changes. There is substantial evidence that their loss may contribute to microvascular instability leading to the formation of microaneurysms, micro-hemorrhages, so-called 'acellular' capillaries, and diminished capillary perfusion in diabetic retinopathy (Arboleda-Velasquez et al., 2015; Kim et al., 2015). Diminution of brain pericytes in both *in vivo* and *in vitro* models of HG exposure is linked to oxidative stress and ROS production (Patrick et al., 2015). Cultured pericytes exhibited signs of oxidative stress and underwent apoptosis in response to HG. Experiments with STZ-induced diabetic mice showed a loss of brain pericyte coverage by 12 week of diabetes.

Pericyte dysfunction can cause BBB injury via several mechanisms including altered synthesis of basement membrane components, reduction of barrier-supporting molecules and secretion of pro-inflammatory factors and MMPs (Persidsky et al., 2016). Intermittent HG,

but not steady HG, increases MCP-1 secretion by pericytes and expression of activating transcription factor 4 and C/EBP homologous protein (CHOP), which act as key mediators of ER stress-associated inflammation and cell death (Zhong et al., 2012).

The effects of AGEs on basement membrane synthesis were investigated in a study utilizing triple-culture of human BMVECs, pericytes and astrocytes (Shimizu et al., 2013). The study highlighted pericytes as important regulators of the BBB basement membrane. AGEs induced basement membrane hypertrophy by enhancing TGF- β secretion, which had autocrine action to promote the production of fibronectin by pericytes (Figure 2). Additionally, the action of TGF- β on BMVECs in conjunction with pericyte-secreted VEGF and MMP-2 played a role in the altered integrity of TJs. AGEs-related changes in pericyte function could be inhibited by RAGE siRNA. Activation of pericyte RAGE led to further expression of the receptor (Wang et al., 2012b). Thus, AGEs increase fibronectin secretion by pericytes and directly induce basement membrane hypertrophy in BBB via RAGE signaling.

In addition to their structural and autocrine/paracrine role, brain pericytes interact extensively with BMVECs, facilitated in part by gap junctions between the two cell types. The down-regulation of the gap junction protein, connexin 43 (Cx43), by siRNA intravitreal injection (Tien et al., 2014) has been linked to the induction of apoptosis in retinal vascular cells. Death of vascular cells and augmented permeability were observed in retinas that received intravitreal injections of Cx43 siRNA. Therefore, Cx43 down-regulation may contribute to the dysfunction of the blood-retina barrier through vascular permeability in diabetic retinopathy. Though this effect is best characterized in retinal tissue, several studies have demonstrated a significant role for Cx43 in regulation of TJ proteins, such as occludin, at the BBB (Chronopoulos et al., 2010), and ZO-1 in kidney (Xie et al., 2013).

Comorbidities associated with DM

The macrovascular complications of DM are considered a serious risk factor for stroke, leading to increased mortality and delayed recovery (Mankovsky and Ziegler, 2004). Vasoreactivity to inhaled CO₂ was abrogated and consecutive administration of a NO donor did not restore the vasoreactivity to initial baseline. mRNA levels of eNOS and nNOS, and protein levels of phospho-eNOS, nNOS, and phospho-nNOS were all substantially decreased, likely exacerbating endothelial and smooth muscle cell dysfunction during stroke. The down-regulation of BBB TJ proteins and decreased expression of angiogenesis factors such as the angiopoietins, Ang-1 and Ang-2, PDGF- β , and TGF- β may also impair the ability of the vascular network to adapt. In mice, permanent focal ischemia increased infarct volume, neurological deficit, and BBB permeability. VEGF α expression was also impaired, indicating delayed angiogenesis (Poittevin et al., 2015).

In addition to preexisting pathophysiology, which may worsen prognosis following stroke, the nature of vascular and inflammatory reaction to stroke may differ in hyperglycemic animals. An exacerbated increase in high-mobility group box 1 and ICAM-1 during ischemia-reperfusion was demonstrated during mild/transient hyperglycemia conditions in a middle cerebral carotid artery occlusion rat model. Immunohistochemistry of cerebral microvessels showed ICAM-1 in the cortex was considerably elevated 3 days after

reperfusion in hyperglycemic versus non-diabetic rats. In addition, Western blot analysis showed that IL-1 β , but not TNF α , was increased after reperfusion in diabetic rats (Ding et al., 2005).

The interactions of DM type 1 and epileptic attacks to induce combined effects on the BBB has been assessed in pentylenetetrazol (PTZ)-induced rats (Yorulmaz et al., 2015). A considerable increase of cortex, cerebellum, and brainstem permeability was found in all groups, consistent with previous studies (TG, 1989; Sahin et al., 2003). Following seizure, ZO-1 and GFAP immunoreactivity was lower in the brain of diabetic rats compared with their non-diabetic counterparts. The serum level of TNF α was considerably increased in diabetes and diabetes plus PTZ groups; serum IL-12 was elevated in all experimental groups. Therefore, diabetes is able to further compromise BBB integrity during epileptic attacks (Yorulmaz et al., 2015).

Roles for metabolic cofactors in DM pathogenesis

The protective actions of thiamine and benfotiamine, a synthetic S-acyl derivative of thiamine, in response to HG damage have been observed in *in vitro* models of the retinal barrier, which exhibits many commonalities with the BBB. Immortalized human retinal pericytes and endothelial cells in non-contact, cell-to-cell contact, and 3D co-culture in Matrigel were established to mimic the retinal microvascular environment (Tarallo et al., 2012). All types of co-cultures were exposed to stable or intermittent HG in conjunction with thiamine or benfotiamine treatment. Consistent HG exposure induced apoptosis and suppressed cell proliferation in non-contact and cell-to-cell contact models, while these same outcomes were only observable in the cell-to-cell contact model with intermittent HG. In all cases where HG-induced damages were observed, administration of thiamine and benfotiamine reversed the detrimental effects. In 3D co-culture, the addition of thiamine or benfotiamine reconstituted proliferation and re-established endothelial-pericyte interaction after disruption by intermittent HG (Peng et al., 2008).

Ascorbic acid (AA), an oxidant and a free radical scavenger also termed vitamin C, participates in cellular detoxification and is involved in the biosynthesis of collagen, catecholamine, and peptide neurohormones. It accumulates in the CNS and levels in the brain are high compared with plasma or other organs. Across the BBB, AA is transported by GLUT-1 (glucose transporter) as dehydroascorbic acid (DHA). Disruption of the delivery of AA to the retina and brain commonly occurs in hyperglycemic conditions. In STZ-induced diabetic rats, [^{14}C]DHA was reduced by 84% (Minamizono et al., 2006).

Additional pathways in DM-mediated BBB injury

RhoA is a member of the small GTPase homolog family and, together with its downstream effector, Rho-associated coiled-coil containing protein kinase 1 (ROCK1), plays a role in BMVECs regulation. Among its functions are control of cellular adherence, migration, proliferation, permeability, and apoptosis. Activation of this pathway is associated with BBB disruption and phosphorylation of claudin-5 and occludin under inflammatory conditions (Persidsky et al., 2006b). Recent studies demonstrated that activation of the RhoA/ROCK1

pathway due to HG in diabetic retinopathy played an essential role in microvascular damage by directly inhibiting TJ protein expression (Lu et al., 2014). Inhibition by siRNA or pharmacological inhibitors could prevent such changes. In the early stages of diabetic neuropathy, HG- and AGEs-mediated vascular damage can be significantly reduced by inhibition of RhoA/ROCK1 by simvastatin (Peng et al., 2008; Peng et al., 2013). Therefore, it follows that ROCK inhibition may have potential in the treatment of insulin-resistant diabetes (Peng et al., 2008).

Conclusion

In spite of the substantial evidence for vascular damage in DM, the role of BBB injury remains under investigation. The unique structure of the BBB suggests that different mechanisms are involved and are distinct from other types of microvasculature such as the retina and peripheral nerves. Oxidative stress and the pro-inflammatory effects of DM significantly contribute to the injury of the brain microvasculature and increased pathologic permeability.

All elements of the NVU, including endothelium, pericytes, basement membrane, astrocytes and perivascular macrophages, are considered to be vital elements assuring proper function and integrity of BBB. Hyperglycemia induces thickening of the basement membrane, increases proliferation of endothelial cells, and promotes permeability. These changes are associated with a greater incidence of encephalopathy and neurodegenerative disease, as well as worsened prognosis in comorbidities such as stroke and epilepsy. Much work remains to be done regarding the mechanistic nature of BBB involvement in DM and potential for therapeutics targeted toward BBB protection with improved neurological outcome.

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Figure 1.

Structural adaptations of the BBB to prolonged hyperglycemia. (A) Components of the BBB under normal physiological conditions. The vessel lumen (red) is enclosed by endothelial cells (blue), which attenuate paracellular transport via TJs. A basement membrane (light green) encloses these components and encases pericytes (orange) associated with the vessel. Astrocytic end feet (green) surround the basement membrane, providing an additional barrier to brain parenchyma access (light blue). (B) Pathologic changes to the BBB following prolonged exposure to hyperglycemia. Capillary density is increased as a result of sprouting from existing microvasculature. The basement membrane exhibits thickening and barrier integrity is decreased, in part due to TJ alteration and increased paracellular diffusion. Lastly, pericytes associated with the vessel undergo degeneration, leading to ‘acellular capillary’ appearance histologically.

Figure 2.

Impact of AGEs on cellular components of the BBB *in vitro*. Shimizu et al. (2013) demonstrated that AGEs may stimulate the production of fibronectin through RAGE and TGF-β, presumably leading to BM thickening. Additionally, claudin-5 expression is reduced in endothelial cells exposed to AGEs, which act through RAGE, VEGF, and MMP-2. Claudin-5 is also reduced by TGF-β exposure, suggesting that pericytes may modulate claudin-5 through this mediator (Shimizu et al., 2013). Given that VEGF, TGF-β, and MMP-2 are secreted proteins, it is assumed that VEGF and TGF-β act in an autocrine/paracrine manner while MMP-2 exerts its enzymatic action extracellularly.