Neurovascular Coupling in Cognitive Impairment Associated With Diabetes Mellitus
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Although it is feared that diabetes-induced cognitive decline will become a major clinical problem worldwide in the future, the detailed pathological mechanism is not well known. Because patients with diabetes have various complications of vascular disease, with not only macrovascular but also microvascular disorders, vascular disorders in the brain are considered to be one of the mechanisms in diabetes-induced cognitive impairment. Indeed, disruption of the blood–brain barrier (BBB) has been observed in some diabetic patients and experimental diabetes models. Moreover, white matter lesions, part of the evidence of BBB dysfunction, are reported to be observed more frequently in patients with diabetes. Animal studies demonstrate that diabetes enhances BBB permeability through a decrease in the level of tight junction proteins and an increase in matrix metalloproteinase activity. However, there are several reports indicating that BBB disruption does not occur with diabetes. Therefore, the association of BBB breakdown with diabetes-induced cognitive impairment is not conclusive. Recently, neuronal diseases involving dementia have been induced experimentally through dysfunction of neurovascular coupling, which involves blood vessels, astrocytes and neurons. Diabetes-induced cognitive decline may be induced via disruption of neurovascular coupling, with not only vascular disorder but also impairment of astrocytic trafficking. Here, the relation between vascular disorder and cognitive impairment in diabetes is discussed. (Circ J 2011; 75: 1042–1048)

Key Words: Blood–brain barrier; Cognitive impairment; Diabetes mellitus; Neurovascular coupling

There is concern worldwide that the number of individuals diagnosed with type 2 diabetes mellitus (T2DM) in 2010 is estimated to be 220 million, and that this will continue to increase. Diabetes induces chronic vascular complications, not only macrovascular disorders such as cardiovascular and cerebrovascular disease, but also microvascular disorders (eg, nephropathy, retinopathy and neuropathy). Recently, epidemiological studies reported that T2DM is also a risk factor for cognitive impairment, indicating that diabetes-induced cognitive decline will become a major clinical problem worldwide in the future. Diabetes-related cognitive dysfunction has been recognized in humans, but the pathogenesis of this condition is still debated. On the other hand, evidence from animal studies demonstrates that altered function of the blood–brain barrier (BBB) could be a potential contributing cause. BBB change is induced by alteration of cerebral microvascular endothelial cell connections, so BBB disruption is a microvascular-associated disease; in other words, a small-vessel disease. Representative cerebral small-vessel diseases evaluated by magnetic resonance (MR) imaging, such as white matter lesions (WMLs), are frequently observed in patients with T2DM compared with non-diabetic patients, indicating that diabetes-induced BBB disruption occurs in clinical practice. Moreover, disorders of the microcirculation also play an important role in inducing cognitive impairment and dementia, suggesting that they should be viewed as neurovascular coupling disorders. We here review diabetes-induced cognitive impairment, focusing on vascular disorders, based on basic and clinical research.

Cognitive Impairment Associated With Diabetes

There are many reports about cognitive decline in T2DM patients, which was first recognized 15 years ago. In a population-based cohort study of 462 men, diabetic patients had a higher risk ratio of cognitive impairment evaluated by the Mini-Mental State Examination (MMSE) [rate ratio=1.23 (1.04–1.46)]. Following that report, many investigators have focused on the association between abnormalities in glucose tolerance and impaired cognitive function. Biessels et al’s systematic review of 14 eligible longitudinal population-based studies demonstrated that the prevalence of dementia, including both Alzheimer disease and vascular dementia, was higher in individuals with T2DM than in those without diabetes. In particular, patients with T2DM have been found to have specific and global cognitive deficits characterized by decreases in verbal memory, nonverbal memory, attention, processing speed and executive function. Overall, it seems that abnormal glucose tolerance is associated with cognitive impairment in humans; however, epidemiological data to date lack

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Neurovascular Unit in DM-Induced Cognitive Decline

sufficient detailed mechanistic studies. Lamport et al comprehensively reviewed cognitive impairment associated with impaired glucose tolerance (IGT) and poor glucose tolerance, and suggested that 2 separate mechanisms should be considered in diabetes-induced cognitive impairment. One is an acute mechanism in which changes in glucose level lead to immediate cognitive decrement. The second is a chronic mechanism in which prolonged and sustained IGT results in damage to the cognitive systems. A very recent paper by Euser et al indicates that despite the impaired cognitive function observed in diabetic patients at baseline, elevated fasting glucose level and insulin resistance were not associated with impaired cognitive function in the elderly without a history of T2DM in either the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER) or the Rotterdam Study. These results suggest that there is a threshold for the effects of dysglycemia and factors other than hyperglycemia, such as diabetic complications, to affect cognitive function; therefore, the mechanisms of diabetes-induced cognitive impairment are complicated. Microvascular disorders, one of the most common diabetic complications, are considered to contribute to diabetes-induced cognitive impairment. We here discuss the relation of cognitive decline to diabetes-induced microvascular disorders such as disruption of the BBB, vascular aging and dysfunction of the neurovascular unit.

**Disruption of the BBB by T2DM**

*Structure of the BBB*

The BBB acts as a complex cellular gate that tightly regulates the transport of molecules into and from the central nervous system (CNS). The BBB contains endothelial cells, pericytes and astrocytic endfeet (Figure 1). Endothelial cells are sealed by tight junctions and adherens junctions that limit the paracellular flow of water, ions and larger molecules into the brain and organize the cell membrane into apical and basal domains. The tight junctions between endothelial cells are maintained by transmembrane proteins such as occludin, claudins and junction adhesion molecules. These proteins are bound to actin-anchored cytoplasmic proteins such as zonula occludens (ZO)-1, ZO-2 and ZO-3. Astrocytes also have an important role in regulation of the BBB. The major features of the endothelial cell phenotype in the BBB are dependent on astrocyte-derived signals, such as transforming growth factor β1, which induces the differentiation of endothelial cells and pericytes, and vascular endothelial growth factor, basic fibroblast growth factor, and glial cell line-derived neurotrophic factor, which are involved in modulation of BBB permeability. On the other hand, pericytes surround the endothelial cells and are able to constrict brain capillaries and change the blood flow. These cell–cell interactions are key features of the maintenance of the BBB.

*Clinical Features*

Palmer presented a schematic representation of the interaction between BBB breakdown, neuroinflammation and neurodegeneration. Neurodegenerative change is exacerbated by the linked process of BBB disruption and neuroinflammatory change; therefore, BBB breakdown is considered to

**Figure 1. Structure of the blood–brain barrier. ZO, zonula occludens.**
be a predictor of neuronal dysfunction. Leakage of albumin into the cerebrospinal fluid (CSF) evaluated by CSF–serum albumin ratio was found in patients with not only established vascular dementia but also Alzheimer disease in 1990. In the late 1990s, Skoog et al demonstrated that the mean CSF–serum albumin ratio was higher in demented than in non-demented individuals at age 85, but was not related to dementia severity. In the 2000s, MR enhancement with the contrast agent gadolinium DTPA contributed to assessment of vascular leakage. Increased permeability of the BBB demonstrated by MR imaging was detected in patients with T2DM aged 65–70 years by Starr et al. Interestingly, brain signal intensity was enhanced, particularly in the basal ganglia. However, few reports have investigated BBB disruption in patients with T2DM. In contrast, Dai et al reported that the BBB is well maintained in diabetic patients. They used postmortem brain tissue from 12 diabetic patients and performed immunohistochemical staining against an endothelium-specific antigen PAL-E, which is a useful cellular vascular marker for the absence or loss of vascular blood–tissue barriers, and 2 plasma proteins, IgG and albumin. They demonstrated no remarkable difference in the staining of PAL-E, IgG and albumin between subjects with and without diabetes. However, the sample number was too small (only 7 patients with T2DM) to reach a clear conclusion on the relation between diabetes and BBB disruption.

WMLs are detected as hyperintense areas on T2-weighted MR images, in areas that are bilaterally and symmetrically sited in the hemispheric white matter. The prevalence of WMLs is significantly related to the risk of stroke, cognitive decline and dementia. The causes of WMLs are not well known, but there is evidence for BBB dysfunction. Moreover, Farrall et al’s systematic review showed that significantly increased BBB permeability was observed with increasing number of WMLs. Manschot et al demonstrated that T2DM patients with more WMLs and brain atrophy had lower performance on neuropsychological tests than controls. They also showed that cognitive function in T2DM patients was inversely related to WMLs, atrophy, and the presence of infarct, and was modestly associated with HbA1c and diabetes duration in those with cognitive impairment. However, the association between diabetes and WMLs remains unanswered because meta-analysis of WMLs has revealed no consistent association between T2DM and the incidence of WMLs. Taken together, these findings indicate that although disruption of the BBB is considered to be more frequent in individuals with cognitive decline and dementia, the association of BBB breakdown with diabetes-induced cognitive impairment is not conclusive in clinical practice.

**Basic Experiments**

In 1986, Lorenzi et al reported that even after a relatively short duration of diabetes, the BBB manifests increased permeability. Huber et al clearly showed time-dependent, molecular weight-dependent failure of barrier function using streptozotocin-induced diabetic rats. In that study, they demonstrated that changes in BBB permeability were region...
specific. The midbrain was most susceptible compared with the hypothalamus, cerebellum, cerebral cortex, hippocampus, basal ganglia, and thalamus. They used Evans blue albumin, [3H]-insulin, and [3H]-sucrose to evaluate the molecule size effect on BBB permeability. Interestingly, total albumin extravasation did not change in diabetic rats compared with controls, indicating that overall the BBB remained intact. However, a significant increase in albumin extravasation was observed in the midbrain and basal ganglia, suggesting that the BBB disruption by diabetes is region specific. Although an association between cognitive impairment and region specific change in BBB is not well known in diabetic models, a contribution of a disrupted BBB in the basal ganglia is reported in the pathogenesis of human immunodeficiency virus type 1 (HIV)-induced dementia involving dopaminergic neurons. On the other hand, Kortekaas et al demonstrated that impaired BBB function is observed in the midbrain of patients with Parkinson’s disease. Recent clinical studies suggest a link between T2DM and Parkinson’s disease, and basic animal experiments indicate a pathogenic link between BBB disruption and degeneration of dopaminergic neurons. These reports suggest that region-specific BBB permeability, especially in the mesolimbic dopamine system, may have a key role in T2DM-induced cognitive impairment.

Although normal aging increases the permeability of the BBB, diabetic rats are more susceptible to nanoparticle-induced cerebrovascular reactions in the brain and neuronal damage. Hawkins et al showed that in experimental rat diabetes, BBB permeability to sucrose increased in parallel to a decrease in the level of the tight junction proteins, occludin and ZO-1. On the other hand, increased plasma matrix metalloproteinase (MMP) activity is implicated in the degradation of BBB tight junction proteins and thus, increased BBB permeability. They also demonstrated that peripheral MMP activity was increased in streptozotocin-induced diabetic rats (Figure 2). In contrast, ATP-binding cassette transporters, such as P-glycoprotein (P-gp, also called ABCB1) and breast cancer resistance protein (Bcrp, also called ABCG2), were highly expressed in the BBB and active-transport proteins exporting substances into blood. Hawkins et al reported that diabetes leads to “upregulation” of efflux transport at the BBB. Moreover, Reichel et al also demonstrated that T2DM does not significantly alter the expression and function of Pgp and Bcrp, using brain capillaries and choroid plexus of streptozotocin-induced diabetic rats. These results suggest that in diabetic rodents, it is mainly components of the BBB that are altered, such as a decrease in the level of tight junction proteins and an increase in MMP activity. As shown in Figure 2, these altered BBB functions may permit enter to blood components such as activated monocytes and inflammatory cells, which induce chemokines and cytokines, and lead to a poincious effect on neurons, resulting in impaired cognitive function.

### Treatment Options in Diabetes-Induced BBB Disruption

In streptozotocin-induced diabetic rats, epinephrine administration led to an increase in microvascular Evans blue (EB)-labeled albumin efflux into the brain, with a decrease in antioxidant enzymes. However, treatment with an angiotensin II type 1 receptor blocker (ARB), candesartan, significantly attenuated this permeability of brain tissue through an antioxidant effect and beneficial effects on vascular endothelial permeability. Moreover, treatment with another ARB, losartan, also significantly attenuated epinephrine-induced EB-labeled albumin transport into brain tissue in diabetic hypertensive rats, indicating that blockade of the renin–angiotensin system (RAS) appears to be effective in preventing diabetes-induced BBB dysfunction. A systematic review of 22 large clinical trials of antihypertensive drugs in over 143,000 non-diabetic patients showed that blockade of the RAS contributed to reducing the risk of developing T2DM, indicating that the RAS is deeply involved in the patho- logical mechanism of diabetes. The RAS is also involved in the pathogenesis of small-artery remodeling in T2DM. On the other hand, it was recently reported that angiotensin II increases cerebral microvasculature inflammation via oxidative stress, leading to greater immune–endothelial interaction and greater BBB permeability. Moreover, Fleegal-DeMotta et al reported that angiotensin II directly modulated transcytotic and paracellular permeability in BBB endothelial cells. These results indicate that diabetes-induced BBB disruption may be partly caused by RAS activation and prevented by RAS blockade.

On the other hand, Manschot et al indicated that T2DM patients treated with 3-hydroxy-3-methylglutarylcoenzyme A (HMG-CoA) reductase inhibitors (ie, statins) showed less severe WMLs evaluated by brain MR imaging. In rodent models, statins show a protective effect on endothelial permeability in diabetes. This beneficial effect is not through inhibition of the cholesterol synthesizing enzyme, HMG-CoA, but through a direct action on the endothelium. For example, statins are reported to prevent the infiltration of mononuclear cells into the CNS, prevent BBB permeability with a reduction in leukocyte migration and prevent adhesion of activated T lymphocytes to pre-activated human brain microvascular endothelial cells (HBMEC). In an in vitro model, statins also attenuated glutamate-induced BBB disruption and enhanced stabilization of the BBB by nitric oxide-dependent dephosphorylation of endothelial myosin light chain. Furthermore, a statin blocked amyloid β-induced proinflammatory reactions in HBMEC. These results indicate that statins can be expected to attenuate diabetes-induced cognitive function via an inhibition of BBB permeability. Therefore, RAS blockade and treatment with statins may prevent BBB permeability, mainly through effects on the endothelium; however, it is not well known whether these approaches are actually effective for diabetic patients, so further clinical investigation is anticipated.

### Vascular Aging in Diabetes

Diabetes enhances organ senescence in diabetes models; for example, senescence-associated molecules such as p21, p27, p53 and Rb were increased in the myocardium of diabetic db/db mice and the increase in these molecules was completely suppressed by treatment with candesartan. Moreover, T2DM may enhance senescence, mainly of the tubule cells of the kidney. Diabetes also enhances vascular senescence, which affects BBB permeability. Previous reports by Bouchard et al suggest that T2DM seems to accelerate the aging process of the vascular wall and that the CNS capillary bed is also a target for diabetic microangiopathy. Recently, Orimo et al clearly demonstrated that treatment with high glucose accelerated senescence of human endothelial cells, with a reduction in the expression of silent information regulator two ortholog 1 (Sirt1). Moreover, a significant decrease in Sirt1 expression was observed in the aorta of streptozotocin-induced diabetic mice compared with non-diabetic mice.
indicating that Sirt1 is involved in diabetes-induced vascular disorders. Interestingly, Takeda et al. recently showed a new relationship between T2DM and Alzheimer disease involving vascular disorder using an established diabetic Alzheimer disease mouse model. They clearly showed that the diabetic condition induces cerebrovascular changes, such as vascular inflammation and cerebral amyloid angiopathy, and results in greater cognitive dysfunction. We previously reported that the main RAS components, angiotensin II and aldosterone, exert cross-talk in vascular smooth muscle cell senescence, with involvement of oxidative stress and Ki-ras2A. Although we have not yet investigated the effect of T2DM in this model, activation of the RAS may be also related to diabetes-induced vascular senescence.

Neurovascular Coupling Disorder in Diabetes

Hemodynamic coupling between neuronal, glial and vascular components links blood flow dynamically to brain activity. The so-called “CNS neurovascular unit” or “neurovascular coupling” is linked to many common human CNS pathological conditions, including dementia. Decreased cerebral blood flow (CBF) induces dysfunction of neurovascular coupling and leads to neuronal injury and death. In contrast, T2DM may cause loss of homeostasis of the cerebral micro-environment, with not only vascular dysfunction but also astrocytic dysfunction. Recent evidence suggests that astrocytes play a significant role in the regulation of local CBF. Astrocytes grown in high glucose show increased oxidative stress. Prolonged hyperglycemia interferes with astrocytic gap junctional communication. Disruption of astrocytic trafficking of metabolites and signaling molecules may alter neurovascular coupling and contribute to the change in brain function in T2DM and result in diabetes-induced cognitive decline (Figure 2).

Conclusion

Diabetes-induced cognitive impairment is one of the most worrying future problems in patients with T2DM. Diabetes-induced microvascular complications, such as disruption of the BBB, are partly related to this cognitive impairment. However, there is insufficient clinical evidence to prove this mechanism. Moreover, it is not easy to evaluate BBB breakdown. In the kidney, microalbuminuria (ie, disruption of the membrane structure in the kidney) is the earliest clinical evidence of diabetic nephropathy, and is a predictive marker of kidney damage. These clinical markers of chronic kidney disease may predict small-vessel disease of the brain. Therefore, in the brain, if there was a similar predictive marker of BBB breakdown, diabetes-induced cognitive impairment could be prevented before irreversible changes occurred. To prevent a decline in the quality of life for diabetic patients, further investigations of this mechanism with both clinical and basic experiments are awaited.

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