The Effects of Diabetes on the Neurovascular Unit in Vascular Cognitive Impairment and Dementia

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ABSTRACT

Vascular cognitive impairment and dementia (VCID) is the second most prevalent form of dementia accounting for ~20% of all dementia diagnoses. Dementia is an umbrella term that initiates mild to severe cognitive impairment in the form of memory loss, confusion, and impaired judgment. With vascular and brain health directly related, the term VCID has been coined to describe effects of vascular dysfunction and vascular disease on brain health and neurodegeneration. The neurovascular unit (NVU) describes the relationship between brain cells and the surrounding cerebral vascular cells. Recent reports Although mechanisms that occur within the NVU are not well understood, they could potentially provide key insight into understanding VCID development and progression. Diabetes is a known vascular risk factor for NVU dysfunction and ischemic stroke. It has been identified that comorbid conditions such as diabetes increase the risks of post-stroke cognitive impairment (PSCI) and worsened outcomes such as VCID. This review article will describe the impact of diabetic induced ischemic injury on brain microvasculature. Recent research has incorporated preclinical VCID models to investigate the connection between diabetes, NVU dysfunction, and cognitive impairment. Specifically, this article will bring much attention to current findings in VCID research in relation to diabetic complications such as impaired cerebral blood flow (CBF), vascular restructuring, and compromised NVU function.

Introduction

VCID is one of the leading causes of physical disability worldwide and is increasing in elderly populations, specifically patients with vascular disease. According to the World Health Organization, 55 million people are currently diagnosed with dementia, with ~10 million new cases each year. Of these cases, about 20% are classified as VCID with Alzheimer's Disease (AD) being diagnosed at a much higher rate[1]. Additionally, VCID shows similar pathologies that are found in AD such as increased amyloid beta (Aβ) and brain atrophy[72]. Therefore, VCID is categorized under AD & Related Dementias (ADRD) with mixed pathologies[73]. Alongside hypertension and obesity, diabetes is one of the leading risk factors of VCID. Previous studies have shown a 73% increased risk of dementia, including a 56% increase of Alzheimer's Disease and 127% increase of vascular dementia, in individuals with diabetes over those without this metabolic disease[2]. Although diabetes is an established risk factor of VCID, the precise pathological relationship between the two diseases is unclear. Diabetes also increases risk of stroke, which blocks blood flow to the brain, causing potential brain injury and VCID in a phenomenon known as PSCI. Due to the connection between diabetes, stroke, neurovascular health, and cognitive impairment, both genetic and lifestyle factors (*e.g.* sleep, diet, and exercise) contribute to an individual's risk of VCID.

The US Centers for Disease Control and Prevention defines a healthy brain as one that can perform all processes necessary for cognition, including learning, judgment, language, and memory. Major determinants of brain

health include genetic/epigenetic, environmental/behavioral, and social factors. These factors can positively or negatively affect brain health. Vascular risk factors, including cerebrovascular disease (*e.g.* small vessel disease, large artery atherosclerosis, and structural and functional injury) and cardiovascular disease (*e.g.* atrial fibrillation, valvular disease, cardiomyopathy) have negative effects on brain health[3]. Diabetes, hypertension, obesity, dyslipidemia, brain trauma, and smoking collectively increase the risk of these cerebrovascular and cardiovascular complications, which ultimately can cause brain damage and ultimately lead to neurological dysfunction and neurodegenerative disease[75]. Furthermore, stroke events and other related cerebrovascular injuries such as micro/macro infarcts and white matter hyperintensities are associated with cognitive impairment and dementia[74]. Due to the link between cardiovascular and brain health, intervention that decreases cardiovascular and stroke risk can also decrease the risk of developing cognitive impairment. Since VCID reflects diverse vascular pathologies, this paper will focus mainly on defining the neurovascular unit and its intricate functions, describing the nature of CBF and vascular remodeling after ischemic incidents, and evaluating the relationship between neurovascular dysfunction and cognitive impairment in diabetes.

A. The Neurovascular Unit: Structure and Function

The NVU is the basic unit of the cerebral vasculature and describes the relationship between neuronal activity and blood flow. The NVU comprises vascular cells (endothelial cells, pericytes, and smooth muscle cells (SMCs), glial cells (astrocytes, microglia, and oligodendroglia), and neurons[4,5]. Capillaries are lined by a semi-permeable endothelial layer known as the blood-brain barrier (BBB), which regulates the transport of solutes and ions between the brain and circulating blood to maintain homeostasis and facilitate neural signaling[6-9]. These endothelial cells are surrounded by pericytes and astrocyte end feet[4] . The NVU acts as the guardian of cerebral homeostasis by promoting proper CBF, maintaining BBB function, and regulating the clearance of toxic waste from the brain[10]. The linkage of neurons with other NVU cells facilitates CBF through two major processes identified as neurovascular coupling and cerebral autoregulation. These specific processes regulate CBF in response to environmental fluctuations and location of metabolic activity, ultimately ensuring that adequate blood flow is directed to brain tissue where needed. Decreased CBF and impaired BBB function are pathophysiological markers of neurodegenerative disease such as VCID[4]. However, our understanding of the nature of the pathological mechanisms that occur in VCID, specifically in the presence of diabetes, is still limited. Below, the various cell types which comprise the NVU are described, including their relationship with diabetes.

A1. Endothelial Cells

Endothelial cells sheath blood vessels and are the epicenter of the BBB. They possess unique luminal and abluminal membranes containing specific enzymes, receptors, and transport proteins that give endothelial cells the ability to selectively allow nutrients in and waste out of brain tissue[10]. The presence of tight junction proteins such as claudin 5 (expressed in all vasculature) and claudin 1, 3, and 12 (expressed specifically in the brain microvasculature) allow for the connection of adjacent endothelial cells within the lining of cerebral blood vessels[6]. Tight junctions also function to block the transport of polar blood solutes (cells and proteins) across the BBB into the brain and are essential for maintaining BBB integrity and cerebral homeostasis [10,11]. The delicate balance maintained by endothelial cells can be disrupted by metabolic disease and ischemic insult. For example, after a stroke event, claudin-5 is targeted for degradation by matrix metalloproteinase, disrupting the BBB and causing neuronal dysfunction, sclerosis, and/or brain edema[12].

Additionally, diabetes-related TNF-α upregulation in the vitreous of diabetic patients and in retinas of diabetic rats increases retinal vascular permeability by altering ZO-1 and claudin-5 tight junction proteins[13]. Aside from tight junctions, endothelial cells produce trophic factors, supporting neuronal survival via vasotrophic coupling between the microvasculature and NVU[14,15]. The activation of toll-like receptor-4 (TLR4) by diabetes or a stroke event

can cause endothelial-mesenchymal transition (EMT), impairing vasotrophic coupling and causing chronic inflammation, glymphatic dysfunction, remodeling, and demyelination, conditions which ultimately result in cognitive impairment [15]. The alteration of tight junction proteins to cause BBB breaches and the disruption of vasotrophic coupling processes to cause TLR4 activation are two illustrations of how diabetes and stroke can target the vascular endothelium to cause cognitive impairment.

A2. Neurons

Neurons are key players in important CBF-regulatory processes, such as neurovascular coupling and cerebral autoregulation. In cerebral autoregulation, neurons, which are highly sensitive to environmental changes such as those in CBF, command vascular responses via neurotransmissions, adjusting vascular diameter with shifts in blood pressure[16]. Similarly, neurons direct neurovascular coupling by regulating CBF regionally, releasing chemical signals to direct blood flow towards areas of higher metabolic activity by inducing vasoconstriction or vasodilation[5]. The ability of neurons to carry out these CBF-regulatory processes is impaired in diabetes. Essential communication between neurons and other NVU cells is inhibited, and furthermore, diabetes-related changes in glucose levels may cause oxidative stress and glucose neurotoxicity, which can cause neuronal damage and death [17,18,19].

A3. Glial Cells

Astrocytes are located between neurons and endothelial cells and mediate interactions between the two[5,10], communicating with endothelial cells through their end feet[4]. Astrocytes are key players in the neurovascular coupling process. Neurons are highly sensitive to minute changes in oxygen and nutrients delivered through CBF, and release neurotransmitters from synapses to adjacent astrocytes. These astrocytes then use chemical signals to relay these changes to endothelial cells, which go on to make the physiological changes involved in neurovascular coupling (i.e. vasoconstriction or dilation). Astrocytes are further recognized for their essential role in the BBB by influencing the expression of tight junction proteins to maintain BBB integrity and by regulating BBB molecular crossings through their involvement with aquaporin 4 water channels and K+ transporters[10,20]. Diabetes-related hyperglycemia alters astrocyte metabolism and inhibits astrocyte proliferation by causing cell cycle arrest at the G2/M phase, disrupting neuron-endothelial communication in CBF-regulatory processes[19].

Microglia are another type of glial cell within the NVU. They are macrophage-like cells with the ability to elicit inflammatory response and partake in several key processes such as trophic support, waste removal, and brain protection/repair[21]. Hyperglycemia can cause tissue stress, leading to microglia hyper-activity and excessive inflammation, which can lead to VCID symptoms.

A4. Pericytes

Pericytes are mural cells that are along cerebral capillaries, embedded within the vascular basement membrane and covering the endothelium[10]. In neurovascular coupling, pericytes interpret changes in cell length to modulate vascular diameter. Pericytes also play a role in BBB development and function[22]. One study demonstrates that pericytes are necessary for BBB formation, and the volume of pericyte coverage of capillaries directly correlates with vascular permeability (because pericytes inhibit the expression of molecules which increase vascular permeability) and immune cell infiltration/neuroinflammation[23]. Additionally, this study shows that pericytes are involved in tight junction formation and vesicle transportation. Pericyte loss is a sign of diabetes-associated microvascular disease, and a decrease in pericyte-to-endothelial ratio has been demonstrated in diabetic versus control models[24,25]. Three different subtypes of pericytes exist: thin strand, mesh, and ensheathing. Thin strand pericytes extend thin projections along capillaries[15,76]. Mesh pericytes form a mesh-like sheath around the entire blood vessel which allows them to contribute to

NVU integrity[15]. Ensheathing pericyte projections surround the entire arteriole. Ensheathing pericytes cover vessels almost completely[15,77]. Due to the presence of alpha-smooth muscle actin (α-SMA), ensheathing pericytes are thought to possess significant contractile properties and are therefore most relevant to CBF regulation [15,76,77].

A5. Basement Membrane (Basal Lamina)

The vascular stability and structural integrity of the NVU is supported by the basement membrane (also known as basal lamina), the extracellular matrix which separates endothelial cells from astrocytes/pericytes, and astrocytes/pericytes from each other[10]. The basement membrane mediates the exchange of signals between these cells, facilitating the vasomodulation necessary to maintain proper CBF[26]. The basement membrane is composed primarily of laminin, type IV collagen, nidogen (glycoprotein), and perlecan (proteoglycan)[27]. Cells and vessels of the NVU anchor themselves within the basement membrane for stability[10]. Furthermore, the basement membrane constitutes the noncellular component of the BBB, playing a vital role in BBB integrity[28]. Diabetes can cause vascular basement membrane thickening surrounding microvessels, leading to poor basement membrane integrity and tight junction degradation which increases BBB permeability[29].

B. Cerebral Blood Flow: Proper Function and Complications in Diabetes, Stroke, and VCID

B1. Cerebral Blood Flow Function

The brain is an organ which requires large amounts of energy to facilitate various functions including maintenance, growth, and more complex functions such as communication, learning, and memory[16,30]. In fact, the brain consumes 20% of a person's total energy output[31]. Lacking its own energy reserves, the brain must rely heavily on continuous blood flow to deliver adequate oxygen and nutrients as well as properly remove waste products. Proper blood flow is essential because neurons and glia are acutely sensitive to even miniscule chemical changes within the brain environment[3]. This is facilitated by the brain's complex, 400-mile-long network of vasculature composed of over 100 billion vessels[16,32], and the NVU, the basic functioning unit of cerebral vasculature which links neurons to vasculature[10]. Proper CBF is highly sophisticated, as blood must reach the correct parts of the brain at specific times and in exact doses[30]. Areas of the brain with higher metabolic activity tend to receive increased CBF[33]. Optimal brain health and CBF maintenance depends on cerebrovascular and cardiovascular health, and vice versa; neurological damage can inhibit neurohumoral mechanisms controlling the heart, vasculature, and metabolism, resulting in cardiac damage and hypertension[3].

B2. Neurovascular Coupling, Cerebral Autoregulation, and Myogenic Response

Key components to CBF regulation include neurovascular coupling (also known as functional hyperemia) and cerebral autoregulation, which both occur within the NVU. Neurovascular coupling describes changes in CBF in response to changes in neuronal activity[34]. Typically, CBF is directed towards more active regions of the brain to meet higher metabolic demand[35]. For example, spatiotemporal studies (which assign a motor, visual, auditive, or cognitive task and measure blood flow) show that finger tapping results in increased CBF to the contralateral motor cortex[35,36]. Neurovascular coupling is largely facilitated by astrocytes: during neuronal activity, the release of neurotransmitters (i.e. glutamate) increase Ca2+ in astrocytes, which then release vasoactive arachidonic acid metabolites through their end feet into blood vessels. Prostaglandin E2 and epoxyeicosatrienoic acids dilate blood vessels in response to hypoxic

conditions, while 20-hydroxyeicosatetraenoic acid constricts vessels in response to hyperoxic conditions[10,17]. Contractile proteins expressed by pericytes, including α-SMA and tropomyosin also contribute to changes in vascular diameter.

Another central process which maintains proper CBF is cerebral autoregulation, which allows cerebral vasculature to maintain consistent blood flow with shifts in blood pressure by modulating vascular diameter (ie. vasoconstriction and vasodilation). The primary process behind cerebral autoregulation is myogenic response, the ability of smooth muscle cells to contract with increased BP or dilate with decreased BP to maintain stable CBF[16]. Myogenic reactivity describes this change in vessel diameter in response to BP changes, and myogenic tone describes the amount of contraction at a particular pressure level[37]. Regulating myogenic tone is the mechanism by which the microvasculature ensures smooth blood flow throughout the brain at different blood pressures[38]. Cerebral autoregulation and myogenic response are regulated by neurons and astrocytes of the NVU, which transmit chemical signals for vasomodulation of arterial smooth muscle cells. Neurons activate astrocytes, pericytes, and other neurons[39]. From there, both neurons and astrocytes secrete prostaglandins, nitric oxide, and adenosine to induce smooth muscle cell vasodilation. Astrocytes further secrete potassium and epoxyeicosatrienoic acids for vasodilation, while secreting arachidonic acid for vasoconstriction[35]. It is also known that the release of compounds including 5-hydroxytryptamine and bradykinin contribute to constriction and dilation respectively[38]. Furthermore, some studies suggest that pericytes around the capillaries may also contribute to vasodilatory effects in microvasculature[40]. Together, these processes ensure each part of the brain receives adequate blood flow, protecting the brain from potentially dangerous fluctuations in blood flow.

B3. CBF in Diabetes and Dementia

It is clinically well established that impaired CBF, commonly caused by diabetes, increases risk for brain injury, malfunction (as in VCID), and in extreme cases, death. Understanding the connection between CBF, diabetes, and dementia is important in creating neuroprotective treatments which can target CBF-related malfunctions following diabetes. Multiple studies, including have found that type 2 diabetes (T2D) disrupts proper CBF[33,41]. Diabetes can inhibit proper CBF in multiple ways. First, it remodels the architecture of large cerebral arteries and results in pathological neovascularization and vascular rarefaction (explained in section C). On a physiological level, it disrupts cerebrovascular regulatory functions, impairing myogenic response, neurovascular coupling, and cerebral autoregulation[16].

Cerebral autoregulation has been found to be impaired after stroke and in people with diabetes and/or hypertension[41]. Additionally, one study utilizing transcranial doppler ultrasonography and continuous blood pressure measurements in stroke victims identified a strong correlation between cerebral autoregulation dysfunction and various subtypes of ischemic stroke[42]. Evidence suggests that diabetes disrupts autoregulation by increasing myogenic tone (and thus vasoconstriction) in response to increased blood pressure[38,43]. Additionally, diabetes may result in hypo- or hyper-sensitivity to chemical vasoconstriction/vasodilation signals. For instance, sensitivity and constriction to 5-hydroxytryptamine were increased while response to bradykinin, a compound which induces SMC contraction and blood vessel dilation) decreases[38]. Brain damage results when autoregulation is inhibited, CBF cannot change in response to BP changes, and the brain's oxygen demands are not met[16]. Although exact mechanisms have not yet been identified, it is hypothesized that stroke events also impair cerebral autoregulation by damaging arterioles and capillaries[42]. Subsequently, the relationship between microvessels and neurons is disrupted, edema formation results from increased endothelial cell permeability and endocytoses, and matrix degradation occurs during hemorrhagic transformation following ischemic stroke, ultimately impairing autoregulation and proper CBF[44].

Neurovascular coupling is another process impaired by diabetes. Neurovascular coupling can be observed in response to light stimulation via light flickering, which increases retinal neural activity[45]. Studies show that flicker-induced vasodilatation in retinal arteries is malfunctional in patients with diabetes, indicating that impaired neurovas-

cular coupling may be associated with diabetic retinopathy[46,47]. In explanation, diabetes results in pericyte and astrocyte loss, two cell types essential for neurovascular coupling (pericytes for their contractile properties and astrocytes for their role facilitating communication between neurons and the surrounding vasculature). Unable to receive and respond to neurotransmitters (i.e. glutamate) during fluctuations in neuronal activity, these cells lose their ability to modulate CBF where necessary.

Collectively, these T2D and stroke-related complications to blood flow can lead to neuronal damage and cognitive impairment via metabolic dysfunction and the creation of hypoxic or hyperoxic conditions. The hippocampus, the brain region responsible for tasks related to memory and cognition, is highly susceptible to hypoxia, and therefore major symptoms of VCID are caused by hypoxic conditions induced by CBF dysfunction[48]. Mild hypoperfusion detrimentally affects protein synthesis, which is necessary for the synaptic plasticity required in learning and memory. More severe hypoperfusion reduces ATP synthesis, limiting the brain's ability to carry out actions as an extremely metabolically active organ[49]. In addition to limiting the supply of oxygen and nutrients, reduced CBF also reduces clearance of carbon dioxide and toxic molecules such as β-amyloid (Aβ) and α-synuclein, allowing for further neurodegeneration[49]. Hypoxic conditions can also alter the pH levels, electrolyte levels, and water gradient within the neuronal milieu, further leading to cerebral damage in the form of edema, white matter lesions, and the accumulation of glutamate and toxic proteins such as Aβ and tau, hallmarks of AD. When CBF reduction exceeds 80%, neurons begin to die[50.]

C. Cerebrovascular Network and Its Components

Metabolic diseases such as diabetes can negatively affect the cerebrovascular architecture through vascular remodeling, pathological neovascularization, vasoregression, and even altering the physiology of blood vessels[16]. These vascular alterations inhibit NVU processes mentioned previously, specifically neurovascular coupling and cerebral autoregulation. This section discusses the organization of the neurovascular network through which the NVU drives blood flow. Understanding the cerebrovascular structure is necessary to develop effective VCID models.

Cerebral arteries deliver oxygenated blood to the brain. The four major cerebral arteries are the right and left internal carotid arteries (ICAs) and the right and left vertebral arteries. ICAs run through the carotid canal and dura mater, dividing into middle and anterior cerebral arteries in the subarachnoid space[7]. They supply 80% of blood to the brain[7,9]. The vertebral arteries run through the vertebral foramina and foramen magnum, forming the basilar artery on the ventral side of the brain stem where it branches into two posterior cerebral arteries[7,15]. The "circle of Willis" describes the point at the base of the skull where branches of the ICA (responsible for anterior circulation) and the vertebral arteries (responsible for posterior circulation) convene[7,16]. A dense web of arteries lines the neural surface[7]. These pial vessels are located within the glia limitans (the outermost layer of brain tissue composed of astrocyte end feet) and pia–arachnoid layers, and are surrounded by cerebrospinal fluid[8,51]. Stemming from these pial vessels are penetrating arteries, smaller arteries which permeate brain tissue and subsequently become parenchymal arterioles[7,8].

There are several differences between pial and parenchymal arteries. First, pial vessels are arranged so that occlusion of one vessel does not significantly affect CBF, whereas penetrating and parenchymal arterioles are largely unbranched and therefore a single occlusion can have major consequences, including impaired CBF and tissue damage[8]. Second, pial arteries are extrinsically innervated and are therefore able to receive external chemical signals for myogenic response, while parenchymal arteries are intrinsically innervated from within the brain tissue, receiving signals released into the perivascular space surrounding the arteriole by subcortical neurons[7,8,16,52]. This intrinsic innervation is likely responsible for vasomotion, the spontaneous oscillation of myogenic tone[7,16,53].

In contrast to macrovasculature that contains larger cerebral arteries, the microvasculature is composed of a dense network of capillaries. Contractility exists at the precapillary arteriole level, facilitated by ensheathing pericytes which receive their contractility abilities through the presence of α-SMA[15]. Capillaries contract and dilate to regulate CBF, sending deoxygenated blood through pial veins and venules, through various sinuses, and eventually back to the

heart (7). Thus, blood travels through three levels to supply oxygen to the brain: (1) surface pial vessels, (2) penetrating arteries, and (3) capillaries[16].

As described above, the cerebrovasculature is responsible for a plethora of functions which maintain homeostasis in the brain. The macrovasculature and microvasculature work in harmony to regulate CBF, ensuring deliverance of oxygen and nutrients to all parts of the brain. The cerebrovasculature is subject to structural changes via neovascularization, vascular wall remodeling, and vasoregression[16]. Cerebrovascular restructuring is a normal process but can cause serious detriment when stemming from metabolic diseases such as obesity and diabetes.

C1. Neovascularization, Vascular Remodeling, and Vasoregression

Understanding vessel growth and formation is important to understanding VCID, which largely stems from inadequate CBF due to diabetes- and stroke-related complications in processes such as cerebral autoregulation and neurovascular coupling and detrimental changes in vasculature. Part B discussed the effects of diabetes on the abilities of vessels to constrict and dilate to sustain proper CBF. Here, the processes which contribute to expansion and reduction of the vasculature itself are named and described.

First, angiogenesis is the process by which new blood vessels are formed from preexisting ones. Angiogenesis is induced through a cascade of events starting with ischemia and subsequent tissue hypoxia. Hypoxic conditions caused by diabetes-induced CBF impairment activate hypoxia inducible factor-1α (HIF-1α), which in turn activates vascular endothelial growth factor-A (VEGF-A) and VEGF receptor 2 (VEGFR-2). Quiescent endothelial tip cells in the blood vessels then migrate to guide a sprouting capillary through the extracellular matrix towards the VEGF-A stimulus[54]. Simultaneously, the proliferation of stalk cells allows the sprouting capillary to elongate. Once the tip cell reaches the source of the VEGF-A stimulus, it merges with another tip cell to create a continuous vessel through which blood can flow.

Arteriogenesis is a second mechanism of vessel growth. Whereas in angiogenesis new capillaries sprout from pre-existing ones, in arteriogenesis collateral arteries develop from pre-existing arterioles. These pre-existing vessels are remodeled by increasing lumen and wall thickness, increasing vessel stability and enhancing delivery of oxygen and nutrients by increasing blood flow 10 to 20 fold[55,56]. Arteriogenesis is a vital process in saving tissues and organs following occlusion of a major artery[56]. Through this growth of collateral vessels, capillaries can become arterioles and arterioles can become larger arteries, meeting increasing demands for oxygen and nutrients. Together, angiogenesis and arteriogenesis contribute to neovascularization, the expansion of blood vessels.

In contrast to angiogenesis which increases capillary density, vasoregression is the process by which capillaries are pruned. This is a normal process during development, trimming down vasculature after initial formation to help the primary vascular plexus reach maturity. However, malfunction in vasoregression is also the first step in microvascular complications. Causes of vasoregression include a) unstable vessel formation and structure due to insufficient signaling during development; b) apoptosis of endothelial cells, usually to remove excess blood vessels during maturation of the vascular plexus; c) damage or inflammation leading to pericyte loss (via apoptosis or migration); and d) malfunction of endothelial tight junctions, which stabilize vessels[57]. The precise underlying mechanisms of vasoregression have yet to be elucidated, although evidence suggests that it occurs through a combination of decreased VEGF expression and increased ANG2 expression. Below, the relation between these vascularization/regression processes and VCID is discussed.

C2. Diabetes, Vascular Injury, and Cognitive Impairment

It is well established that people who are diabetic and undergo a stroke event are more likely to develop vascular dementia and have worsened functional outcomes. In fact, VCID develops in about 30% of stroke survivors within 3 months of a stroke event[58]. However, this number varies across countries and diagnostic methods. One study in the Netherlands on 176 patients found a 70% PSCID rate over a three-month period, while another study in South Korea

with 620 patients found a rate of 69.8% after 3 months[59,60]. Risk factors of PSCID include hypertension, hyperlipidemia, atrial fibrillation, smoking, and diabetes, which we will focus on in this section. In addition to increasing risk of cognitive impairment, diabetes also increases severity of cognitive impairment especially after ischemic stroke[61]. Cerebrovascular remodeling associated with diabetes can hamper neuronal repair and contribute to cognitive decline.

Several studies have been conducted on the effects of diabetes on neovascularization and cognition using diabetic animal models. For example, one study found increased neovascularization encompassing both angiogenesis and arteriogenesis in T2D Goto-Kakizaki (GK) rats increased vascular volume and surface area, capillary density, and vascular permeability due to diabetes-related impairment of BBB integrity[62]. Glycemic control (via treatment with metformin) prevented neovascularization. Another study seeking to elucidate the effects of diabetes on hippocampal neurovascular remodeling and the resulting PSCI in vivo found that stroke in diabetic Wistar rats causes increased neovascularization and resulted in more severe cognitive impairment[61]. Cognition (measured through a novel object recognition (NOR) task) and sensorimotor function (assessed through an adhesive removal test) both experienced greater deficits after stroke in diabetic over control rats. Overall, these two studies suggest a strong correlation between diabetes, neovascularization, and cognitive impairment.

Evidence shows the impaired integrity of cerebral blood vessels in rats with diabetes who have undergone a stroke event[63]. The disrupted BBB allows blood to leak into the brain after ischemic stroke, a phenomenon known as hemorrhagic transformation. In Wistar rats, hemorrhagic transformation was shown to be greater in diabetic over control rats[61]. Interestingly, more severe hemorrhagic transformation was noted in female over male rats. Excessive neovascularization coupled with ischemic injury increases risk of hemorrhagic transformation, which results in poor stroke recovery, including PSCID. Hemorrhagic transformation also promotes vasoregression. Studies with diabetic GK rats showed a dramatic decrease in cerebrovasculature in diabetic animals, but not control animals, after stroke[64]. There are several proposed mechanisms for this post-stroke vasoregression, including apoptosis, nitrative stress, and ferroptosis[64,65].

In summary, diabetes impairs processes which contract/dilate vasculature to maintain proper CBF, resulting in hypoxic conditions. This triggers pathological neovascularization in the brain, a highly metabolic organ which relies on high oxygen consumption to operate. Because the newly formed vasculature lacks structural integrity, a stroke event (for which diabetes is a risk factor) may lead to hemorrhagic transformation, ultimately promoting vascular rarefaction and poor stroke recovery, including augmented cognitive deficits.

D. The Implications of Neuroprotective Therapies in VCID

According to the 2020 National Diabetes Statistics Report, more than 34 million adults (13.0% of all US adults) have diabetes[66]. This review has established the increased risk of VCID in diabetics due to dysfunction within the NVU and cerebrovascular architecture. With skyrocketing diabetes prevalence, there is a need for therapies which address complications of diabetes (like VCID), in addition to therapies designed to prevent diabetes in the first place. As a result, therapeutics are being developed which address diabetes-related neurovascular complications for the prevention of VCID in diabetics. This article will discuss two emerging neuroprotective therapies: 1) Endothelin receptor blockers to address decreased CBF resulting from cerebrovascular dysfunction, and 2) iron chelators to combat iron deposition in the brain stemming from compromised endothelial integrity.

In patients with diabetes/VCID, impaired vasoconstriction and vasodilation causes improper CBF and is a common form of cerebrovascular dysfunction. Endothelin-1 (ET1), a vasoactive peptide in the endothelin family, is a potent vasoconstrictor[67]. Increased levels of ET1 circulating in the brain are risk factors for cerebrovascular disease[68]. ET1 impairs the ability of the NVU to regulate CBF, and in the case of breached BBB (as in diabetes and VCID), ET1 can reduce CBF. Increased levels of ET1 have been shown to mediate pericyte constriction in Alzheimer's disease, restricting CBF. ET1 inhibits proper myogenic response and tone in diabetes and causes neurovascular uncoupling, thus impairing the two key processes for CBF regulation[68,69]. This conclusion was supported by Palmer et al, who

showed a direct correlation between tissue hypoxia and ET1 levels in dementia patients (ALcendor). ET1 can also promote cerebrovascular remodeling and pathological cerebral neovascularization. ET receptor agonists, such as BQ3020, block ET1 activity, improving stroke outcomes and preventing cognitive impairment by preventing/reversing pathological cerebral angiogenesis and restoring myogenic tone and neurovascular coupling[69,70]. Thus, ET1 is a promising therapeutic target for treating VCID (as well as other forms of cognitive impairment) in diabetic patients.

Iron chelators are another emerging neuroprotective therapy that targets the vascular complications of diabetes and VCID. Impaired endothelial integrity and hemorrhagic transformation cause bleeding in the brain, resulting in elevated levels of circulating free iron and iron deposits. Iron accumulation is common in many neurodegenerative diseases[67]. Iron accumulation becomes detrimental due to its neurotoxicity and may cause oxidative stress or cell death[71]. Iron chelators such as deferoxamine (DFX) prevent the accumulation of excess iron and post-stroke DFX treatment has been shown to prevent both vasoregression and PSCI in diabetes (Li et al. and Abdul et al).

Overall, ET1 and iron chelation inhibition target different neurovascular complications resulting from diabetes (i.e., dysregulation of CBF and neuronal bleeding, respectively), but both are postulated to achieve favorable neuroprotective effects. Thus, using our understanding of the mechanistic aspects of VCID to guide the development of novel neuroprotective interventions has yielded promising results in treating diabetic patients after stroke.

Conclusion

Instances of VCID and PSCI are increasing worldwide, especially due to the rising prevalence of vascular disease such as diabetes which increases risk of stroke and cognitive impairment. A properly functioning NVU and structurally sound neurovasculature are paramount to maintaining cerebral homeostasis, including regulating CBF and maintaining proper BBB function. Our knowledge of the mechanisms of VCID underscore the importance of healthy neurovasculature in brain health and cognitive function. Diabetes and stroke impact cognitive function through their effects on the NVU. First, diabetes disrupts CBF regulation by interfering with processes such as neurovascular coupling, cerebral autoregulation, and myogenic response. With the brain's lack of natural energy reserves and acute sensitivity to changes in CBF, these complications contribute to hypoxic conditions which are highly detrimental to brain health and cognitive abilities. Second, the ability of diabetes and stroke events to alter cerebral microvasculature via pathological neovascularization, vascular remodeling, post-stroke vasoregression, and hemorrhagic transformation, can further deteriorate vascular structure and induce cognitive impairment.

The relationship between diabetes related microvascular complications and VCID is a newly emerging but increasingly significant area in the field of neurodegenerative disease. Various forms of microvascular dysfunction (impaired cerebral autoregulation, myogenic uncoupling, and BBB disruption, etc.) are being targeted by neuroprotective therapies such as ET1-receptor blockers and iron chelators. Although recent breakthroughs in this field are encouraging, there is still much we have yet to understand. The correlation between diabetes and VCID is well established, but we have yet to elucidate the precise pathological relationship between the two diseases, including the exact molecular mechanisms by which they affect processes such as cerebral autoregulation, neurovascular coupling, and post-hemorrhagic transformation vasoregression. A comprehensive understanding of these mechanisms will be the basis for the discovery of more potent and effective therapies which can prevent these disruptions to CBF and the cerebral microvasculature, allowing the NVU to maintain its homeostasis and the brain to drive normal cognitive processes like learning and memory.

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