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Blood–Brain Barrier Disruption, Vascular Impairment, and Ischemia/Reperfusion Damage in Diabetic Stroke

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Stroke is a major cause of death and long-term disability accompanied by steep social and medical costs. Diabetes mellitus (DM) is a chronic, lifelong, severe metabolic health problem characterized by hyperglycemia, attributable to insulin deficiency, insulin resistance, or a combination of both. Patients with DM suffer from massive vascular damage and rapidly develop microvascular and macrovascular diseases, often leading to end organ damage such as damage to the kidneys, eyes, and peripheral nervous system.[1] DM raises the risk of cardiovascular and cerebrovascular diseases (such as ischemic stroke) multifolds.[2] Approximately 30% of stroke patients have DM,[2] and stroke in diabetic patients follows a specific clinical pattern mostly resulting in poor prognosis.[3,4] DM alters metabolism, and complicates stroke pathology, making it challenging to treat the diabetic ischemic brain. Long-term functional recovery in patients with DM-stroke is often hindered by increased predisposition to recurrent strokes.[5] As a result, neurological deficits and fatality rates are significantly higher in patients with stroke who also have DM.[6] Experimental studies have shown that DM instigates a cascade of events leading to severe vascular dysfunction, hemorrhage, earlier and more severe white matter injury, and aggravated inflammatory responses compared with non-DM ischemic brain.[7–9] In this article, we review diabetic stroke–induced blood–brain barrier (BBB) disruption, vascular and reperfusion damage, as well as underlying pathophysiological mechanisms such as shear stress, diminished fibrinolytic capacity, mitochondrial dysfunction, and oxidative stress as well as neuroinflammation in mediating diabetic stroke–induced vascular and reperfusion damage.

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Blood–Brain Barrier Disruption in Diabetic Stroke

DM triggers an assortment of vascular pathologies including increased vascular permeability, which contributes to high morbidity of ischemic stroke.[6] The BBB is composed of endothelial cells, astrocytic end-feet, pericytes, and a thick basement membrane, and serves as a dynamic semipermeable barrier separating the peripheral circulation and the central nervous system.[10,11] The BBB is essentially a diffusion barrier that allows the passage of hydrophobic molecules and metabolic products by passive diffusion while preventing the entry of microscopic substances, hydrophilic molecules, and potential neurotoxins.[10,11] Between the cerebral endothelial cells, tight junctions (TJs) form a diffusion barrier and prevent bloodborne substances from entering the brain.[10] Astrocytic end-feet continuously line all cerebral vessels and are crucial for maintaining the TJ barrier.[10] Prolonged DM induces thickening of the capillary basement membrane, which then increases BBB permeability because of alterations in the physical dimensions of the meshwork and changes in the normal electrical charge surrounding the pores between endothelial cells.[12] A ruptured BBB is permeable to large molecules and facilitates the invasion of inflammatory factors, neurotoxins, and pathogens into the brain.[11] BBB disruption occurs in the acute phase after stroke and is among the initial steps that precede many neurological disorders.[13] BBB permeability has also been associated with an increase in endothelial transcytosis.[14] In experimental stroke, it has been shown that hemorrhagic transformation and BBB permeability usually increases within 7 days poststroke in non-DM animals, but it can extend up to 14 days or longer in DM stroke animals.[14–16] Systemic inflammation has been shown to worsen BBB disruption and exacerbate functional deficits after stroke in mice.[17] In a vicious cycle, while BBB disruption allows the entry of inflammatory factors into the brain, the pro-inflammatory factors in turn promote BBB disruption and lead to hemorrhagic transformation in diabetic stroke animals.[16,18]

Cerebral edema is a pathological increase of brain water content leading to a rise in the intracranial pressure and may occur because of swelling of brain cells, called cytotoxic edema or from BBB disruption and an increase interstitial water content called vasogenic edema.[19,20] Immediately following ischemic injury, cytotoxic cerebral edema may ensue, driven by impaired cellular metabolism and dysfunction of sodium and potassium membrane ion pumps, which leads to increased water uptake and subsequent swelling of brain cells.[20] Vasogenic edema formation is driven by hydrostatic pressure gradient, which is influenced by intracranial pressure, systemic blood pressure, capillary occlusion, and vasospasm.[19] In vasogenic edema, following TJ and BBB rupture, transendothelial permeability increases, facilitating the entry of water and plasma proteins such as albumin and immunoglobulin G into the brain interstitial space.[21] The osmotic pressure gradient mediated by osmotically active molecules such as Na^+ and proteins also mediates water influx in vasogenic edema.[19] Poststroke brain edema was found to be associated with greater neurological deficits both during admission and at discharge.[22] In addition, a higher incidence of poststroke edema was reported in patients with DM.[22] Similarly, in type 2 diabetes mellitus (T2DM) mice subjected to stroke, a significant increase in both ipsilateral and contralateral brain water content was observed.[23] Additional complications such as hypertension can exacerbate cerebral edema after stroke in patients with DM.[23] Understanding the mechanism and consequences of BBB disruption after stroke and in particular diabetic stroke are essential to developing therapeutics.[24,25]

Role of Tight Junction

TJs between adjacent endothelial cells seal interendothelial cell gaps, while adheren junctions (AJs) maintain interendothelial cell contact.[26] The TJs create a high endothelial electrical resistance and low paracellular permeability, thereby preventing ion flux and paracellular diffusion across the BBB.[26] During ischemic stroke, TJ degradation occurs in a multistep time-dependent fashion involving a number of interdependent signaling mechanisms.[11] During the reperfusion phase of ischemia–reperfusion (I/R) injury, reactive hyperemia and loss of cerebral autoregulation lead to acute opening of the BBB TJs.[11] The stability of the TJ is maintained by anchoring TJ proteins (eg, occludin and claudin) and the AJ protein cadherin to the actin cytoskeleton via multiple accessory proteins such as zonula occludens.[26,27] Under normal conditions, the actin–myosin cytoskeleton is distributed as short filaments and diffuse monomers between the endothelial cells.[27] However, when subjected to hypoxic stress, the actin filaments polymerize into linear stress fibers and the actin–myosin cytoskeleton contracts via myosin light chain phosphorylation, leading to increased cytoskeletal tension, weakening of the junction seals, and increased BBB permeability.[27,28] Stroke decreases TJ transmembrane proteins such as occludin, claudin, zonula occludens, and junction adhesion molecules.[11,26] DM decreases TJ proteins in the parenchymal blood vessels, inducing BBB disruption, and facilitates extravasation of albumin and inflammatory factors into the brain.[29] Calcium is critical to maintain intercellular junctions; hence, an increased intracellular calcium after stroke can also alter the expression of TJ and AJ proteins in the brain, affecting BBB permeability.[26] T2DM significantly induces brain TJ disruption and exacerbates BBB damage, increases infarct volume, as well as worsens neurological deficits in mice with stroke.[30]

Role of Pericytes

Pericytes are functional components of the neurovascular unit that are embedded in the basement membrane and closely interact with endothelial cells of capillaries and venules via physical contact and paracrine signaling.[31] Pericytes have contractile properties, inductive, structural, and regulatory roles, and their interactions with endothelial cells are crucial for BBB maintenance, and structure and function of basement membrane and endothelial TJs.[32,33] During ischemic stroke, constriction and death of pericytes leads to prolonged cerebral blood flow (CBF) decrease and BBB disruption.[34] Hyperglycemia in DM causes oxidative stress and depletes pericytes from the cerebral microvasculature, which then leads to BBB disruption.[35] During the hypoxic phase of stroke, pericyte migration from their usual microvascular location can directly or indirectly induce BBB disruption.[32] Following ischemic stroke, pericytes acquire multipotent stem cell activity, leave the vessel wall, proliferate, and exhibit microglial cell phenotype.[36,37] Pericyte-derived vascular endothelial growth factor (VEGF) may promote BBB disruption after stroke in mice.[38] VEGF is an important growth factor for angiogenesis and directly stimulates endothelial cell proliferation and migration.[39] Although VEGF is beneficial when administered before stroke[40] or at a delayed time point after stroke,[41] in the acute phase of stroke, VEGF increases BBB leakage, cerebral hemorrhage, and infarction volume.[41] DM rats suffer from vascular damage, which is aggravated after an ischemic insult; the effects of VEGF may be exacerbated in DM stroke rats compared with nondiabetic stroke rats.[42,43] In DM, mitochondrial oxidative stress leads to both endothelial cell damage as well as pericyte depletion and BBB leakage.[44] In the minutes to hours following ischemia, endothelial swelling and pericytes mediate capillary constriction, which is followed by rapid pericyte death leading to irreversible constriction of capillaries and BBB damage.[34,45]

Role of Astrocytes

The key functions of astrocytes include participation in BBB formation, maintaining ion and water homeostasis, releasing neurotrophic factors and waste clearance from the brain.[46,47] Astrocyte–endothelial cell interactions have also been shown to be essential in regulating brain water content and electrolyte balance under normal and pathological conditions.[48,49] Ischemic injury to the brain activates astrocytes, and reactive astrocytes can exert a deleterious role (secrete proinflammatory cytokines, inhibit axonal regeneration, infarct expansion) in the acute phase after stroke while exerting a protective role (neurite sprouting, synapse formation, rebuild BBB, secrete neurotrophic factors) in the chronic phase after stroke.[50,51] Using a model of forebrain ischemia in rats, it has been reported that diabetic hyperglycemia suppresses ischemia-induced astrocyte activation, increases astrocyte cell death, damages the astrocyte end-foot lining around cerebral vessels, and the damaged astrocytes exhibit increased withdrawal of the astrocyte end-foot from the cerebral vessel wall.[52,53] Astrocytic end-feet continuously line all cerebral blood vessels and the water channel protein Aquaporin-4 is highly localized to astrocyte end-feet.[47] DM regulates Aquaporin-4 expression in the retina and induces diabetic retinopathy.[54] Knockdown of Aquaporin-4 exacerbates retinopathy by increasing retinal vascular permeability, retinal thickness, and expression of pro-inflammatory factors.[54] Middle-aged rats induced with DM exhibit a significant decrease of paravascular Aquaporin-4 expression in the hippocampus.[55] The interaction between the astrocytes, Aquaporin-4, and endothelial cells regulates brain water content as well as poststroke edema resolution (ie, transport of water via bulk flow from the brain parenchyma to the vascular, intraventricular, and subarachnoid compartments).[49,56] Aquaporin-4 has been implicated in water uptake into the brain tissue during the evolution of cytotoxic edema, as well as in water clearance after vasogenic edema.[21,49,57] Using astroglial conditional Aquaporin-4 knockout mice, it has been shown that deletion of Aquaporin-4 decreases about 30% of brain water uptake after systemic hypo-osmotic stress without affecting BBB impermeability to macromolecules.[49] Another study using global Aquaporin-4-deficient mice has shown that stroke in Aquaporin-4-deficient mice results in improved neurological function as well as ≈30% decrease in cerebral cytotoxic edema compared with control wild-type mice subject to stroke.[57] In contrast, in a freeze-injury model of vasogenic brain edema, Aquaporin-4-deficient mice suffer from worse neurological outcome, greater intracranial pressure and brain water content, indicating that Aquaporin-4 may be required for fluid clearance in vasogenic brain edema.[56] Hence, during the early phase of ischemia, Aquaporin-4 inhibition could facilitate attenuation of cytotoxic edema formation, while during the late phase of ischemic stroke, increased expression of functional Aquaporin-4 could facilitate the reabsorption of vasogenic edema.[58]

Vascular Impairment in Diabetic Stroke

DM induces endothelial dysfunction including impaired blood vessel tone, platelet activation, leukocyte adhesion, thrombogenesis, and inflammation.[59] Impaired insulin signaling in endothelial cells in patients with DM decreases vasodilator nitric oxide and increases vasoconstrictor endothelin-1, resulting in vasoconstriction of blood vessels and decreased CBF.[59] Increased inactivation of nitric oxide and/or decreased reactivity of the smooth muscle to nitric oxide can also lead to decreased vasodilation.[60] Prolonged exposure to such conditions can lead to endothelial dysfunction and atherosclerosis.[60] Arterial stiffness is a commonly encountered complication of DM, which is also associated with poor functional outcome after stroke.[61] DM-induced oxidative stress and inflammatory responses in turn accelerate atherosclerosis.[62] Impaired vasodilation leads to prolonged CBF decrease, which can trigger neuronal cell death.[63,64] Therefore, CBF regulation and the re-establishment of functional microvasculature via angiogenesis and arteriogenesis in the ischemic penumbra help maintain neural function, and create a hospitable microenvironment for neuronal plasticity leading to functional recovery.[63,64]

Angiogenesis is a process of growth of new blood vessels from pre-existing vessels, and has been associated with long-term functional improvement in stroke patients.[65,66] Angiogenesis is typically at a maximum 7 days after stroke, and stroke patients with a higher cerebral blood vessel density have higher survival rates and better functional improvement.[67] DM induces vigorous but dysfunctional angiogenesis and neovascularization; thus, it is associated with poor vessel wall maturity and a large number of nonperfused vessels.[7,16,43] DM rats subjected to a model of transient stroke exhibit a dramatic decrease in vascular volume and surface area along with vascular regression at 14 days after stroke in both the ipsilateral and contralateral hemispheres.[43] Angiogenic responses in DM rodents are mediated in part by an increased VEGF angiogenic signal, increased Angiopoietin-2 (Ang-2) and decreased Ang-1, as well as increased DM-induced oxidative stress.[43,68] Ang-1 promotes vascular maturation and stabilization as well as in increases angiogenesis after stroke.[69] Ang-1 is a primary physiological ligand for TIE2 and plays a vital role in the migration, adhesion, and survival of endothelial cells and in vessel maturation.[70] Ang-1 regulates the organization and maturation of new blood vessels, and decreases leakage and endothelial death.[71] Decreased Ang-1 is related to increased BBB leakage and

brain hemorrhagic transformation after stroke in DM mice.[30] Ang-1 also inhibits pro-inflammatory mediators such as tumor necrosis factor-α, and interleukins (IL-6, IL-8)[72] that exacerbate vascular and white matter damage after stroke in diabetic populations.[9,16]

White Matter Damage in Diabetic Stroke

Compared with gray matter, white matter of the brain is more sensitive and susceptible to ischemic stress because of its relatively limited blood supply.[73] In addition, DM can induce white matter damage, as well as aggravate white matter injury after stroke.[73] DM stroke patients are prone to developing earlier and exacerbated white matter hyperintensities compared with non-DM patients.[74] Vascular dysfunction including BBB disruption that leads to leakage of serum components into the white matter can also induce white matter damage.[75] The white matter in the brain is also highly sensitive to inflammatory responses, which can injure the white matter directly as well as indirectly by damaging the BBB and/or creating an inhospitable environment for axonal/myelin regeneration.[76] Poststroke white matter damage has been associated with poor neurological outcome, small vessel disease, higher risk of recurrent stroke, cardiac complications, and increased mortality.[77] In elderly patients, cardiac diastolic dysfunction is correlated to the severity of cerebral white matter lesions.[78]

White matter remodeling after stroke involves neurogenesis, oligodendrogenesis, and synaptogenesis[76], all of which are adversely affected after stroke in animals with DM.[9] DM decreases neuronal dendrite outgrowth and neuronal cell survival and increases cell death of myelin-producing oligodendrocytes.[9] In the diabetic brain, ischemia induces significant white matter rarefaction, decreases axon and myelin density, and decreases the proliferation and survival of oligodendrocytes progenitor cells, and delays remyelination.[53,79–81] Loss of myelin and axon density hinders the conduction of nerve signals and interneuronal communications affecting sensorimotor functions.[76] Oligodendrocytes are at an increased risk of damage from ischemia, since white matter has limited blood supply when compared with gray matter and there is very little collateral blood flow in deep white matter.[82] Loss of myelin is of primary concern upon oligodendrocyte damage, as injured oligodendrocytes can no longer produce myelin.[76] Both stroke and DM cause axonal and white matter damage, which induce long-term disability because of the limited capacity of the brain for axonal regeneration and its inhibitory environment for axon regrowth, sprouting, and remyelination.[83]

Patients with DM are also highly susceptible to silent strokes, silent white matter injury, and lacunar infarctions in the brain that can trigger cognitive deficits.[84,85] Patients with T2DM frequently develop cognitive dysfunction over time, which has been attributed to vascular pathology, subcortical atrophy, as well as the disruption of white matter integrity and topological organization of the cortical white matter network.[86,87] Since white matter damage can significantly affect cognitive abilities of patients with DM and diabetic stroke, protecting and treating the white matter are critical for long-term improvement of patients.

Ischemia/Reperfusion Damage in Diabetic Stroke

Reperfusion injury is the tissue damage that ensues when blood supply is restored after a period of ischemia. Thrombolysis and mechanical recanalization are major reperfusion strategies after stroke. The only US Food and Drug Administration–approved treatment for ischemic stroke is thrombolysis using tissue plasminogen activator (tPA) that can break down the blood clot and restore blood flow to the brain. However, this treatment is challenged by practicality because of its narrow treatment window (3–4.5 hours after stroke onset). Recent advances in stroke treatments have enabled rapid and effective recanalization using endovascular approaches such as mechanical thrombectomy, and patients with salvageable tissues benefit from improved outcomes.[88] However, only a small population of stroke patients is eligible for acute endovascular intervention.[88] Following I/R, a brief episode of hyperperfusion is followed by secondary hypoperfusion during which there is additional injury to the brain.[89] Oxygen and nutrient deficit during ischemia increases metabolic need of the ischemic penumbra and creates a microenvironment in which the restoration of CBF leads to secondary thrombosis, inflammation, and oxidative stress leading to secondary tissue damage and expansion of infarct volume beyond the initial ischemic insult.[89]

The detrimental effects of DM on I/R injury have been reported in both human and animal studies.[90–93] I/R injury in DM rats subject to stroke increases susceptibility to vascular damage, BBB disruption, and hemorrhage because of the prolific and dysfunctional angiogenesis present in the diabetic rat brain.[90] In T2DM rats, prolonged exposure to ischemic injury aggravates BBB disruption and leads to cerebral edema and hemorrhagic transformation characterized by bleeding into the brain parenchyma, which worsen stroke outcome.[90] Reperfusion to ischemic brain tissue can lead to hemorrhagic infarct conversion, particularly when large infarcts are involved.[94] I/R injury–associated metabolic alterations, energy depletion, and acidosis further damage cerebral vasculature and make them leaky, allowing for entry of edema fluid and red blood cells extravasation.[94] To protect

the microcirculation, small vessel resistance increases and large vessel resistance decreases after stroke in the early phase; however, this may contribute to decreased reperfusion and worse outcome after I/R injury.[95] In T2DM rodents, I/R injury induces worse neurological outcome, higher mortality, large infarct area, ipsilateral hemispheric swelling, and edema.[91]

Mechanisms of Ischemia/Reperfusion Damage in Diabetic Stroke

Effect of Shear Stress in Diabetic Stroke

Shear stress is essentially the tangential force exerted by blood flow on the endothelial cell surface.[96] Shear stress helps create an endothelial transport barrier between blood and underlying tissues while promoting BBB integrity in the brain.[96,97] The internal carotid artery, which branches out from the common carotid artery, is a major blood-supplying vessel to the brain and serves as a low-resistance pathway for CBF autoregulation.[98] Patients with DM exhibit accelerated atherosclerosis and increased susceptibility to arterial thrombosis, which have been attributed to increased shear stress–induced platelet activation, adhesion, and aggregation on the subendothelium.[99] Shear stress and laminar blood flow are atheroprotective.[97] Hence, nonlaminar blood flow in vessels induces alterations in endothelial gene expression, cytoskeletal arrangement, wound repair, leukocyte adhesion, as well as damage to the vasoreactive, oxidative, and inflammatory states of the vessel wall.[97] In addition, plasma von Willebrand factor levels are elevated in the diabetic population, and high shear stress–induced platelet activation is strictly dependent on plasma von Willebrand factor.[99] The naïve blood vessels or damaged cerebral blood vessels that are unable to withstand the shear stress of I/R injury and reperfusion result in rupture and bleeding, thereby aggravating tissue damage caused by ischemic stroke.[90] However, the mechanisms of DM-increased I/R injury after stroke are not very clear.

High Insulin Resistance

Ninety percent to 95% of patients with DM are T2DM.[100] Insulin resistance is commonly present in patients with T2DM, hypertensive patients, elderly and obese populations, and in patients suffering from other vascular diseases.[101] In T1DM rats subject to stroke, treatment with tPA increased brain hemorrhage, BBB permeability, and failed to improve neurological functional outcome while increasing inflammatory responses.[102,103] Another study reported that hyperglycemia during tPA infusion after stroke in rats increased BBB permeability and intracerebral hemorrhage, which may be mediated by oxidative stress mechanisms such as increasing superoxide production by nicotinamide adenine dinucleotide phosphate oxidase.[104] High levels of insulin resistance have been associated with poor outcomes in patients with stroke receiving tPA,[92] and DM stroke patients treated with tPA often have poor prognosis, poor functional outcome, and elevated rates of intracerebral hemorrhage.[92,93]

A large number of patients with DM suffer from large vessel atherothrombotic stroke, and it has been reported that treatment with tPA in such cases is less efficient compared with other types of stroke.[105] Insulin resistance remains a high risk factor for stroke and a potential target for stroke prevention and control.[101]

Experimental studies have reported that insulin resistance caused by defects in glycogen synthesis triggers detrimental changes in the vascular beds.[106] In rats with DM, the infarct volume after stroke was found to be directly proportional to increasing levels of hyperglycemia.[106] Insulin resistance has been reported to mediate hindlimb I/R damage in rats via inflammatory responses that result in microglial activation and neuronal apoptosis.[107] The release of pro-inflammatory adipokines such as IL-6, tumor necrosis factor-α, and plasminogen activator inhibitor-1 (PAI-1) by visceral adipose tissue can initiate the development of insulin resistance and endothelial dysfunction.[108] Accumulated fat and enhanced adipose tissue–derived PAI-1 influence metabolism and vessels in relation to macrophage infiltration, chronic inflammation, and free fatty acid release in obese states.[109] Also, impaired fibrinolysis, which is common among diabetic subjects, is related to insulin resistance, as discussed below.

Diminished Fibrinolytic Capacity

Fibrinolysis is the process of breaking down a fibrin clot (or blood clot) by dissecting the fibrin mesh at several points.[110] The resulting fragments are typically cleared by proteases or by the kidney and liver.[110] DM is associated with diminished fibrinolytic capacity, increased coagulability, and increased concentration in blood of PAI-1.[111] In DM, insulin resistance and metabolic abnormalities are induced during proinflammatory responses by elevated PAI-1 levels involving several cytokines and chemokines.[109] Decrease in fibrinolytic capacity has been implicated in the development and progression of atherosclerotic plaque.[112] As noted above, DM stroke patients often have large vessel atherothrombotic stroke for which tPA therapy has lower efficacy.[105] In a trial that studied patients treated with tPA within 3 hours of stroke onset, elevated serum glucose and DM were found to be independent predictors of intracerebral hemorrhage.[113] Circulating thrombin-activatable fibrinolysis inhibitor and PAI-1 are causal factors for thrombolytic failure. PAI-1 levels are significantly increased in T2DM patients with atherothrombotic ischemic stroke, and increased PAI-1 levels can persist even at

6 months after stroke.[114] Using an ischemic stroke model in mice, it has been shown that targeting of PAI-1 and thrombin-activatable fibrinolysis inhibitor exerts neuroprotective effects by decreasing deposition of fibrin and improving reperfusion.[115]

Mitochondrial Dysfunction and Oxidative Stress

Mitochondrial dysfunction after stroke may result from impaired delivery of glucose and oxygen to brain tissue, mismatch of ATP production and uptake, and from the alterations to mitochondrial properties caused during I/R.[116] Mitochondrial oxidative stress with an increased abundance of reactive oxygen species (ROS) after I/R is a key mediator of diabetic, stroke, and diabetic stroke pathologies.[44] ROS generated from mitochondria mediate neurodegeneration and apoptotic signaling pathways after stroke. DM can aggravate hemorrhagic transformation after stroke via alterations to mitochondrial functions such as decreased cell proliferation, ATP content, mitochondrial membrane potential, and increased matrix metalloproteinase activity, as well as through mitochondrial morphological alterations such as fragmentation, vacuolation, and cristae disruption.[117]

ROS and reactive nitrogen species act as signaling molecules of growth factors to promote angiogenesis; however, according to the "redox window" concept, a mild and not severe level of ROS/reactive nitrogen species stimulates functional angiogenesis.[68] Extracellular glucose has a profound effect on the cellular response to oxidative stress. A level of oxidative stress normally anabolic may be pathological in high glucose conditions.[118] DM in humans and animals exhibits chronic oxidative stress likely because of the metabolism of excess substrates such as glucose and fatty acids that are available in the hyperglycemic state, and because of mitochondrial dysfunction associated with insulin resistance.[12] Hyperglycemia induces oxidative stress, and both upregulate matrix metalloproteinase-9 activity. Increased matrix metalloproteinase-9 expression and proteolytic activation promote BBB damage and brain hemorrhage after stroke in rats.[119] Oxidative stress appears to be a key link between inflammation and angiogenesis and has been reported to be an important factor in the development of necrosis and apoptosis after stroke, particularly upon I/R.[120]

Inflammatory Responses

Pro-inflammatory cytokines increase the production and activity of free radicals and ROS, creating oxidative stress that is detrimental to brain repair.[121] Following an ischemic stroke, mild inflammation can be favorable for brain repair during the chronic stage.[122] However, in the acute phase, when there is uncontrolled inflammation, the activated microglia, astrocytes, and macrophages can exacerbate damage and/or death to the injured brain by releasing pro-inflammatory factors and by creating an inhospitable environment for neural repair.[123] Both the innate and adaptive immune systems are activated upon inflammation; and DM, stroke, and DM-stroke can regulate immune response.[124] DM induces chronic inflammatory responses including increasing inflammatory mediators that can activate neutrophils and vascular endothelium.[91] During I/R, adherence of activated neutrophils to the injured endothelium induces additional damage to the microvasculature and surrounding brain tissue.[91] In experimental T2DM stroke, following I/R, exaggerated neutrophil adhesion in the cerebral microcirculation occurs early after reperfusion alongside aggravated inflammatory responses and poor neurological outcome.[91] Acute increase of inflammatory mediators such as high-mobility group box 1 (HMGB1) in the serum has also been implicated in the inflammatory cascade following DM and I/R injury[125] and is discussed in the following sections. In T2DM patients, a decrease in the production of nitric oxide (vasodilator) and an increase in the secretion of endothelin-1 (vasoconstrictor) enhance vasoconstriction and stimulate release of pro-inflammatory cytokines.[12]

Many inflammatory factors such as inflammatory cytokines that activate NF-κB, matrix metalloproteinase,[9] Toll-like receptors, and receptor for advanced glycation end products are typically increased in animals with diabetic stroke.[8] Toll-like receptors play a primary role in regulating the innate immune response and impact endothelial cell survival and angiogenic responses. HMGB1 is an inflammatory mediator secreted upon injury by immune cells or injured cells. HMGB1 promotes inflammatory responses by stimulating the expression of cytokines such as interleukins (IL-1β, IL-6), and inflammation-related enzyme inducible nitric oxide synthase, which can cause secondary injury to the brain after I/R.[125] HMGB1 release can trigger an inflammatory cascade and binds to its receptors Toll-like receptors 4 and receptor for advanced glycation end products. Receptor for advanced glycation end products has been implicated in the pathogenesis of diabetic complications, inflammatory disorders, and neurodegenerative diseases.[126] Receptor for advanced glycation end products expression is significantly increased in diabetic stroke animals.[8] It has been reported that in cerebral ischemia, HMGB1 triggers matrix metalloproteinase-9 increase in neurons and astrocytes mainly through Toll-like receptors-4.[127]

As a part of tissue response to I/R injury, the expression of several protein kinases is altered such the calcium/calmodulin-dependent protein kinase II, mitogen-activated protein kinase, family members c-Jun N-terminal kinase, extracellular signal-regulated kinase, protein kinase B, and protein kinase C.[128] However, it is unclear whether the changes in protein

kinase expression mediate I/R injury or are activated by ischemia,[128] and discussing the role of all these protein kinases is beyond the scope of this review.

Hyperglycemia in Patients With Acute Stroke

Approximately 30% of patients with acute stroke suffer from either pre-existing DM or newly diagnosed DM.[2,129] Mortality rates are significantly increased in hyperglycemic patients with ischemic stroke.[130] In patients with stroke with normal glucose levels, mortality rates are ≈29%.[130] However, in hyperglycemic patients with stroke with or without a history of DM, mortality rates are increased to 45% and 78%, respectively.[130] Several studies have indicated that in a majority of patients with acute stroke with admission hyperglycemia, hyperglycemia may be triggered by stress responses in reaction to the extensive brain injury.[130,131] Experimental evidence suggests that hyperglycemia significantly worsens both cortical intracellular brain acidosis and mitochondrial function in the ischemic penumbra, while provoking anaerobic metabolism, lactic acidosis, and free radical production.[129,132] While most experimental studies as well as clinical trials have concluded that DM and hyperglycemia are associated with poor outcome poststroke, there are, however, a few reports indicating that hyperglycemia may also extend mild protection against neuronal damage.[133,134] Using 2 experimental stroke models in rodents, it has been shown that the adverse effects of hyperglycemia in stroke may critically depend on several factors: (1) the extent of collateral blood supply to the injured brain tissue, (2) the extent of local CBF decrease, and (3) the timing of hyperglycemia.[134] In the ischemic border tissue, hyperglycemia increases glucose supply and promotes anaerobic metabolism converting glucose to lactic acid.[132,134] Hence, in ischemic regions receiving collateral blood flow, acidosis leads to the expansion of infarct by recruiting the ischemic penumbra into the infarction.[132,134] Therefore, as expected, in animals subject to stroke with diffuse infarctions in the cortex and subcortical brain, acute hyperglycemia significantly increased neurological deficits and infarction volume.[135] However, in lacunar infarction that involves the occlusion of a deep penetrating vessel, hyperglycemia decreases neurological functional deficits without affecting, or even decreasing, subcortical infarction volume.[134,135] These effects are widely attributed to an intact BBB, which prevents entry of neurotoxins and inflammatory factors into the ischemic brain, and poor collateral circulation in the subcortical areas, which makes glucose levels inconsequential.[135,136] In normoglycemic rats subject to stroke, the ischemic border zone exhibits an increased glucose utilization rate with damage to neurons, while in hyperglycemic rats subject to stroke, normal glucose metabolism and structurally intact neurons were observed in the ischemic border zone.[133] Such neuroprotection after stroke in hyperglycemic conditions may be attributable to inhibition of the metabolically demanding, spontaneous, recurrent, and transient increases in

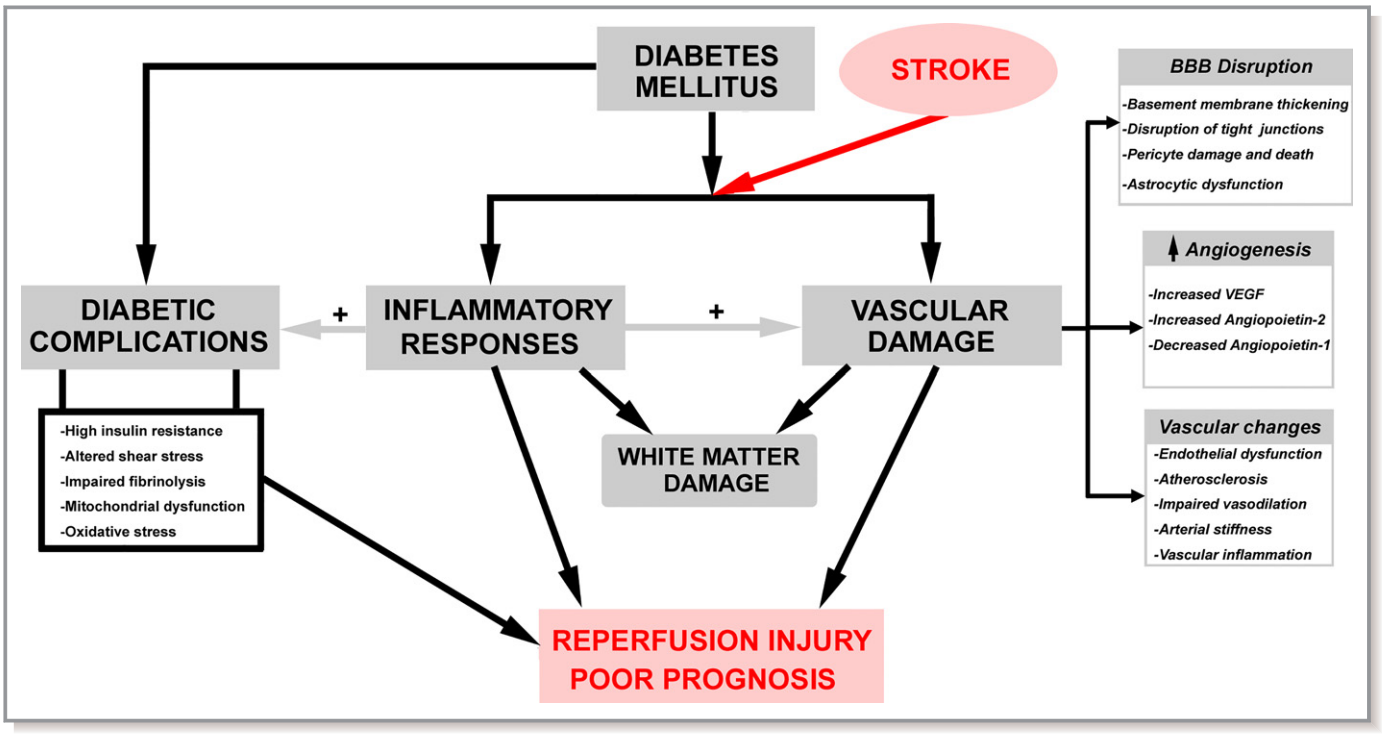

Figure. Summary of pathophysiological cascade in diabetic stroke. BBB indicates blood–brain barrier; VEGF, vascular endothelial growth factor.

extracellular potassium in the cortical ischemic border zone that is observed under normoglycemic stroke conditions.[134] In fact, the very high energy demands imposed by the spreading depression near ischemic core regions may be fueled under high glucose conditions.[136] Persistent hyperglycemia after stroke is an independent determinant of infarct expansion via recruiting the ischemic penumbra and leads to worse functional outcome,[137] hence treatment strategies to normalize glucose levels after stroke are necessary.

Summary

The major pathophysiological changes and underlying mechanisms for stroke in the diabetic population have been discussed and are summarized in Figure. DM exacerbates neurological deficits after stroke and aggravates stroke pathology. DM induces massive microvascular and macrovascular changes, promotes BBB dysfunction, leads to white matter damage, and aggravates inflammatory responses after stroke, thereby, in concert, creating an inhospitable environment for brain repair. Treating the diabetic stroke brain is a challenge, and it is of prime research interest to develop and test treatment strategies specifically for diabetic stroke. Largely, several stroke treatments that are successful in preclinical studies have failed to translate to the clinic. Among the various reasons attributed to this failure is the lack of including comorbidities in experimental design while testing therapeutics. Chronic diseases such as DM can have a profound impact on multiple organs, which is also important to take into account when treating diabetic stroke. Recently, the role of exosomes and MicroRNA in mediating vascular and I/R damage after stroke has emerged.[76,138,139] However, discussions about these mechanisms are beyond the scope of this article. In addition, understanding the acute events, I/R damage, and role of insulin resistance following stroke in diabetic populations may enable development of preventative strategies of secondary stroke. From a research point of view, a comprehensive understanding of the pathophysiological changes after stroke in the diabetic population is necessary for developing successful treatment strategies for stroke patients with DM.

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Disclosures

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References

1. Members WG, Lloyd-Jones D, Adams RJ, Brown TM, Carnethon M, Dai S, De Simone G, Ferguson TB, Ford E, Furie K, Gillespie C, Go A, Greenlund K, Haase N, Hailpern S, Ho PM, Howard V, Kissela B, Kittner S, Lackland D, Lisabeth L, Marelli A, McDermott MM, Meigs J, Mozaffarian D, Mussolino M, Nichol G, Roger VL, Rosamond W, Sacco R, Sorlie P, Stafford R, Thom T, Wasserthiel-Smoller S, Wong ND, Wylie-Rosett J; Committee obotAHAS, Subcommittee SS. Heart disease and stroke statistics—2010 update: a report from the American Heart Association. *Circulation*. 2010;121:e46–e215.
2. Mast H, Thompson JL, Lee SH, Mohr JP, Sacco RL. Hypertension and diabetes mellitus as determinants of multiple lacunar infarcts. *Stroke*. 1995;26:30–33.
3. Megherbi SE, Milan C, Minier D, Couvreur G, Osseby GV, Tilling K, Di Carlo A, Inzitari D, Wolfe CD, Moreau T, Giroud M; European BSoSCG. Association between diabetes and stroke subtype on survival and functional outcome 3 months after stroke: data from the European BIOMED Stroke Project. *Stroke*. 2003;34:688–694.
4. Ergul A, Hafez S, Fouda A, Fagan SC. Impact of comorbidities on acute injury and recovery in preclinical stroke research: focus on hypertension and diabetes. *Transl Stroke Res*. 2016;7:248–260.
5. Callahan A, Amarenco P, Goldstein LB, Sillesen H, Messig M, Samsa GP, Altafullah I, Ledbetter LY, MacLeod MJ, Scott R, Hennerici M, Zivin JA, Welch KM. Risk of stroke and cardiovascular events after ischemic stroke or transient ischemic attack in patients with type 2 diabetes or metabolic syndrome: secondary analysis of the Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) trial. *Arch Neurol*. 2011;68:1245–1251.
6. Yong M, Kaste M. Dynamic of hyperglycemia as a predictor of stroke outcome in the ECASS-II trial. *Stroke*. 2008;39:2749–2755.
7. Li PA, Gisselsson L, Keuker J, Vogel J, Smith ML, Kuschinsky W, Siesjo BK. Hyperglycemia-exaggerated ischemic brain damage following 30 min of middle cerebral artery occlusion is not due to capillary obstruction. *Brain Res*. 1998;804:36–44.
8. Ye X, Chopp M, Liu X, Zacharek A, Cui X, Yan T, Roberts C, Chen J. Niaspan reduces high-mobility group box 1/receptor for advanced glycation end-products after stroke in type-1 diabetic rats. *Neuroscience*. 2011;190:339–345.
9. Chen J, Cui X, Zacharek A, Cui Y, Roberts C, Chopp M. White matter damage and the effect of matrix metalloproteinases in type 2 diabetic mice after stroke. *Stroke*. 2011;42:445–452.
10. Abbott NJ, Patabendige AA, Dolman DE, Yusof SR, Begley DJ. Structure and function of the blood-brain barrier. *Neurobiol Dis*. 2010;37:13–25.
11. Sandoval KE, Witt KA. Blood-brain barrier tight junction permeability and ischemic stroke. *Neurobiol Dis*. 2008;32:200–219.
12. Dokken BB. The pathophysiology of cardiovascular disease and diabetes: beyond blood pressure and lipids. *Diabetes Spectr*. 2008;21:160–165.
13. Shimizu F, Kanda T. [Disruption of the blood-brain barrier in inflammatory neurological diseases]. *Brain Nerve*. 2013;65:165–176.
14. Reeson P, Tennant KA, Gerrow K, Wang J, Weiser Novak S, Thompson K, Lockhart KL, Holmes A, Nahirney PC, Brown CE. Delayed inhibition of VEGF signaling after stroke attenuates blood-brain barrier breakdown and improves functional recovery in a comorbidity-dependent manner. *J Neurosci*. 2015;35:5128–5143.
15. Belayev L, Busto R, Zhao W, Ginsberg MD. Quantitative evaluation of blood-brain barrier permeability following middle cerebral artery occlusion in rats. *Brain Res*. 1996;739:88–96.
16. Chen J, Ye X, Yan T, Zhang C, Yang XP, Cui X, Cui Y, Zacharek A, Roberts C, Liu X, Dai X, Lu M, Chopp M. Adverse effects of bone marrow stromal cell treatment of stroke in diabetic rats. *Stroke*. 2011;42:3551–3558.
17. Denes A, Ferenczi S, Kovacs KJ. Systemic inflammatory challenges compromise survival after experimental stroke via augmenting brain inflammation, blood- brain barrier damage and brain oedema independently of infarct size. *J Neuroinflammation*. 2011;8:164.
18. Borlongan CV, Glover LE, Sanberg PR, Hess DC. Permeating the blood brain barrier and abrogating the inflammation in stroke: implications for stroke therapy. *Curr Pharm Des*. 2012;18:3670–3676.
19. Durward QJ, Del Maestro RF, Amacher AL, Farrar JK. The influence of systemic arterial pressure and intracranial pressure on the development of cerebral vasogenic edema. *J Neurosurg*. 1983;59:803–809.
20. Rosenberg GA. Ischemic brain edema. *Prog Cardiovasc Dis*. 1999;42:209–216.
21. Stokum JA, Gerzanich V, Simard JM. Molecular pathophysiology of cerebral edema. *J Cereb Blood Flow Metab*. 2016;36:513–538.

22. Dostovic Z, Dostovic E, Smajlovic D, Ibrahimagic OC, Avdic L. Brain edema after ischaemic stroke. *Med Arch*. 2016;70:339–341.
23. Tureyen K, Bowen K, Liang J, Dempsey RJ, Vemuganti R. Exacerbated brain damage, edema and inflammation in type-2 diabetic mice subjected to focal ischemia. *J Neurochem*. 2011;116:499–507.
24. Yan T, Venkat P, Chopp M, Zacharek A, Ning R, Roberts C, Zhang Y, Lu M, Chen J. Neurorestorative responses to delayed human mesenchymal stromal cells treatment of stroke in type 2 diabetic rats. *Stroke*. 2016;47:2850–2858.
25. Pardridge WM. The blood-brain barrier: bottleneck in brain drug development. *NeuroRx*. 2005;2:3–14.
26. Brown RC, Davis TP. Calcium modulation of adherens and tight junction function: a potential mechanism for blood-brain barrier disruption after stroke. *Stroke*. 2002;33:1706–1711.
27. Shi Y, Zhang L, Pu H, Mao L, Hu X, Jiang X, Xu N, Stetler RA, Zhang F, Liu X, Leak RK, Keep RF, Ji X, Chen J. Rapid endothelial cytoskeletal reorganization enables early blood–brain barrier disruption and long-term ischaemic reperfusion brain injury. *Nat Commun*. 2016;7:10523.
28. Hicks K, O'Neil RG, Dubinsky WS, Brown RC. TRPC-mediated actin-myosin contraction is critical for BBB disruption following hypoxic stress. *Am J Physiol Cell Physiol*. 2010;298:C1583–C1593.
29. Hoffman WH, Stamatovic SM, Andjelkovic AV. Inflammatory mediators and blood brain barrier disruption in fatal brain edema of diabetic ketoacidosis. *Brain Res*. 2009;1254:138–148.
30. Cui X, Chopp M, Zacharek A, Ye X, Roberts C, Chen J. Angiopoietin/Tie2 pathway mediates type 2 diabetes induced vascular damage after cerebral stroke. *Neurobiol Dis*. 2011;43:285–292.
31. Cai W, Liu H, Zhao J, Chen LY, Chen J, Lu Z, Hu X. Pericytes in brain injury and repair after ischemic stroke. *Transl Stroke Res*. 2017;8:107–121.
32. Liu S, Agalliu D, Yu C, Fisher M. The role of pericytes in blood-brain barrier function and stroke. *Curr Pharm Des*. 2012;18:3653–3662.
33. Hamilton NB, Attwell D, Hall CN. Pericyte-mediated regulation of capillary diameter: a component of neurovascular coupling in health and disease. *Front Neuroenergetics*. 2010;2:5. DOI: 10.3389/fnene.2010.00005.
34. Hall CN, Reynell C, Gesslein B, Hamilton NB, Mishra A, Sutherland BA, O'Farrell FM, Buchan AM, Lauritzen M, Attwell D. Capillary pericytes regulate cerebral blood flow in health and disease. *Nature*. 2014;508:55–60.
35. Patrick P, Price TO, Diogo AL, Sheibani N, Banks WA, Shah GN. Topiramate protects pericytes from glucotoxicity: role for mitochondrial CA VA in cerebromicrovascular disease in diabetes. *J Endocrinol Diabetes*. 2015;2:1–7.
36. Ozen I, Deierborg T, Miharada K, Padel T, Englund E, Genove G, Paul G. Brain pericytes acquire a microglial phenotype after stroke. *Acta Neuropathol*. 2014;128:381–396.
37. Sakuma R, Kawahara M, Nakano-Doi A, Takahashi A, Tanaka Y, Narita A, Kuwahara-Otani S, Hayakawa T, Yagi H, Matsuyama T, Nakagomi T. Brain pericytes serve as microglia-generating multipotent vascular stem cells following ischemic stroke. *J Neuroinflammation*. 2016;13:57.
38. Bai Y, Zhu X, Chao J, Zhang Y, Qian C, Li P, Liu D, Han B, Zhao L, Zhang J, Buch S, Teng G, Hu G, Yao H. Pericytes contribute to the disruption of the cerebral endothelial barrier via increasing VEGF expression: implications for stroke. *PLoS One*. 2015;10:e0124362.
39. Hoeben A, Landuyt B, Highley MS, Wildiers H, Van Oosterom AT, De Bruijn EA. Vascular endothelial growth factor and angiogenesis. *Pharmacol Rev*. 2004;56:549–580.
40. Zechariah A, ElAli A, Doeppner TR, Jin FY, Hasan MR, Helfrich I, Mies G, Hermann DM. Vascular endothelial growth factor promotes pericyte coverage of brain capillaries, improves cerebral blood flow during subsequent focal cerebral ischemia, and preserves the metabolic penumbra. *Stroke*. 2013;44:1690–1697.
41. Zhang ZG, Zhang L, Jiang Q, Zhang R, Davies K, Powers C, Bruggen Nv, Chopp M. VEGF enhances angiogenesis and promotes blood-brain barrier leakage in the ischemic brain. *J Clin Invest*. 2000;106:829–838.
42. Kolluru GK, Bir SC, Kevil CG. Endothelial dysfunction and diabetes: effects on angiogenesis, vascular remodeling, and wound healing. *Int J Vasc Med*. 2012;2012:918267.
43. Prakash R, Li W, Qu Z, Johnson MA, Fagan SC, Ergul A. Vascularization pattern after ischemic stroke is different in control versus diabetic rats: relevance to stroke recovery. *Stroke*. 2013;44:2875–2882.
44. Price TO, Eranki V, Banks WA, Ercal N, Shah GN. Topiramate treatment protects blood-brain barrier pericytes from hyperglycemia-induced oxidative damage in diabetic mice. *Endocrinology*. 2012;153:362–372.
45. Fernandez-Klett F, Potas JR, Hilpert D, Blazej K, Radke J, Huck J, Engel O, Stenzel W, Genove G, Priller J. Early loss of pericytes and perivascular stromal cell-induced scar formation after stroke. *J Cereb Blood Flow Metab*. 2013;33:428–439.
46. Takano T, Oberheim N, Cotrina ML, Nedergaard M. Astrocytes and ischemic injury. *Stroke*. 2009;40:S8–S12.
47. Iliff JJ, Wang M, Liao Y, Plogg BA, Peng W, Gundersen GA, Benveniste H, Vates GE, Deane R, Goldman SA, Nagelhus EA, Nedergaard M. A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid β. *Sci Transl Med*. 2012;4:147ra111.
48. Venkat P, Chopp M, Chen J. New insights into coupling and uncoupling of cerebral blood flow and metabolism in the brain. *Croat Med J*. 2016;57:223–228.
49. Haj-Yasein NN, Vindedal GF, Eilert-Olsen M, Gundersen GA, Skare O, Laake P, Klungland A, Thoren AE, Burkhardt JM, Ottersen OP, Nagelhus EA. Glial-conditional deletion of aquaporin-4 (Aqp4) reduces blood-brain water uptake and confers barrier function on perivascular astrocyte endfeet. *Proc Natl Acad Sci USA*. 2011;108:17815–17820.
50. Li L, Lundkvist A, Andersson D, Wilhelmsson U, Nagai N, Pardo AC, Nodin C, Stahlberg A, Aprico K, Larsson K, Yabe T, Moons L, Fotheringham A, Davies I, Carmeliet P, Schwartz JP, Pekna M, Kubista M, Blomstrand F, Maragakis N, Nilsson M, Pekny M. Protective role of reactive astrocytes in brain ischemia. *J Cereb Blood Flow Metab*. 2008;28:468–481.
51. Li Y, Liu Z, Xin H, Chopp M. The role of astrocytes in mediating exogenous cell-based restorative therapy for stroke. *Glia*. 2014;62:1–16.
52. Jing L, Mai L, Zhang J-Z, Wang J-G, Chang Y, Dong J-D, Guo F-Y, Li PA. Diabetes inhibits cerebral ischemia-induced astrocyte activation—an observation in the cingulate cortex. *Int J Biol Sci*. 2013;9:980–988.
53. Jing L, He Q, Zhang JZ, Li PA. Temporal profile of astrocytes and changes of oligodendrocyte-based myelin following middle cerebral artery occlusion in diabetic and non-diabetic rats. *Int J Biol Sci*. 2013;9:190–199.
54. Cui B, Sun JH, Xiang FF, Liu L, Li WJ. Aquaporin 4 knockdown exacerbates streptozotocin-induced diabetic retinopathy through aggravating inflammatory response. *Exp Eye Res*. 2012;98:37–43.
55. Zhang L, Chopp M, Zhang Y, Xiong Y, Li C, Sadry N, Rhaleb I, Lu M, Zhang ZG. Diabetes mellitus impairs cognitive function in middle-aged rats and neurological recovery in middle-aged rats after stroke. *Stroke*. 2016;47:2112–2118.
56. Papadopoulos MC, Manley GT, Krishna S, Verkman AS. Aquaporin-4 facilitates reabsorption of excess fluid in vasogenic brain edema. *FASEB J*. 2004;18:1291–1293.
57. Manley GT, Fujimura M, Ma T, Noshita N, Filiz F, Bollen AW, Chan P, Verkman AS. Aquaporin-4 deletion in mice reduces brain edema after acute water intoxication and ischemic stroke. *Nat Med*. 2000;6:159–163.
58. Zador Z, Stiver S, Wang V, Manley GT. Role of aquaporin-4 in cerebral edema and stroke. *Handb Exp Pharmacol*. 2009;190:159–170.
59. Hadi HAR, Suwaidi JA. Endothelial dysfunction in diabetes mellitus. *Vasc Health Risk Manag*. 2007;3:853–876.
60. Chen R, Ovbiagele B, Feng W. Diabetes and stroke: epidemiology, pathophysiology, pharmaceuticals and outcomes. *Am J Med Sci*. 2016;351:380–386.
61. Lee Y-B, Park J-H, Kim E, Kang C-K, Park H-M. Arterial stiffness and functional outcome in acute ischemic stroke. *J Cerebrovasc Endovasc Neurosurg*. 2014;16:11–19.
62. Aronson D, Rayfield EJ. How hyperglycemia promotes atherosclerosis: molecular mechanisms. *Cardiovasc Diabetol*. 2002;1:1.
63. Pratt PF, Medhora M, Harder DR. Mechanisms regulating cerebral blood flow as therapeutic targets. *Curr Opin Investig Drugs*. 2004;5:952–956.
64. Plate KH. Mechanisms of angiogenesis in the brain. *J Neuropathol Exp Neurol*. 1999;58:313–320.
65. Arenillas JF, Sobrino T, Castillo J, Davalos A. The role of angiogenesis in damage and recovery from ischemic stroke. *Curr Treat Options Cardiovasc Med*. 2007;9:205–212.
66. Navarro-Sobrino M, Rosell A, Hernandez-Guillamon M, Penalba A, Boada C, Domingues-Montanari S, Ribo M, Alvarez-Sabin J, Montaner J. A large screening of angiogenesis biomarkers and their association with neurological outcome after ischemic stroke. *Atherosclerosis*. 2011;216:205–211.
67. Wei L, Erinjeri JP, Rovainen CM, Woolsey TA. Collateral growth and angiogenesis around cortical stroke. *Stroke*. 2001;32:2179–2184.
68. Abdelsaid M, Prakash R, Li W, Coucha M, Hafez S, Johnson MH, Fagan SC, Ergul A. Metformin treatment in the period after stroke prevents nitrative stress and restores angiogenic signaling in the brain in diabetes. *Diabetes*. 2015;64:1804–1817.
69. Zacharek A, Chen J, Cui X, Li A, Li Y, Roberts C, Feng Y, Gao Q, Chopp M. Angiopoietin1/Tie2 and VEGF/Flk1 induced by MSC treatment amplifies

angiogenesis and vascular stabilization after stroke. *J Cereb Blood Flow Metab*. 2007;27:1684–1691.

70. Suri C, Jones PF, Patan S, Bartunkova S, Maisonpierre PC, Davis S, Sato TN, Yancopoulos GD. Requisite role of angiopoietin-1, a ligand for the TIE2 receptor, during embryonic angiogenesis. *Cell*. 1996;87:1171–1180.
71. Brindle NP, Saharinen P, Alitalo K. Signaling and functions of angiopoietin-1 in vascular protection. *Circ Res*. 2006;98:1014–1023.
72. Wang YQ, Song JJ, Han X, Liu YY, Wang XH, Li ZM, Tzeng CM. Effects of angiopoietin-1 on inflammatory injury in endothelial progenitor cells and blood vessels. *Curr Gene Ther*. 2014;14:128–135.
73. Yu XF, Song RR, Jiaerken Y, Yuan LX, Huang PY, Lou M, Jiang Q, Zhang MM. White matter injury induced by diabetes in acute stroke is clinically relevant: a preliminary study. *Diab Vasc Dis Res*. 2017;14:40–46.
74. Heo JH, Lee ST, Kon C, Park HJ, Shim JY, Kim M. White matter hyperintensities and cognitive dysfunction in Alzheimer disease. *J Geriatr Psychiatry Neurol*. 2009;22:207–212.
75. Wallin A, Sjogren M, Edman A, Blennow K, Regland B. Symptoms, vascular risk factors and blood-brain barrier function in relation to CT white-matter changes in dementia. *Eur Neurol*. 2000;44:229–235.
76. Chen J, Venkat P, Zacharek A, Chopp M. Neurorestorative therapy for stroke. *Front Hum Neurosci*. 2014;8:382.
77. Leys D, Englund E, Del Ser T, Inzitari D, Fazekas F, Bornstein N, Erkinjuntti T, Bowler JV, Pantoni L, Parnetti L, De Reuck J, Ferro J, Bogousslavsky J. White matter changes in stroke patients. Relationship with stroke subtype and outcome. *Eur Neurol*. 1999;42:67–75.
78. Masugata H, Senda S, Goda F, Yamagami A, Okuyama H, Kohno T, Hosomi N, Imai M, Yukiiri K, Noma T, Kohno M. Cardiac diastolic dysfunction is associated with cerebral white matter lesions in elderly patients with risk factors for atherosclerosis. *Tohoku J Exp Med*. 2008;216:99–108.
79. Yatomi Y, Tanaka R, Shimada Y, Yamashiro K, Liu M, Mitome-Mishima Y, Miyamoto N, Ueno Y, Urabe T, Hattori N. Type 2 diabetes reduces the proliferation and survival of oligodendrocyte progenitor cells in ischemic white matter lesions. *Neuroscience*. 2015;289:214–223.
80. Chen J, Ning R, Zacharek A, Cui C, Cui X, Yan T, Venkat P, Zhang Y, Chopp M. MiR-126 contributes to human umbilical cord blood cell-induced neurorestorative effects after stroke in type-2 diabetic mice. *Stem Cells*. 2016;34:102–113.
81. Venkat P, Chopp M, Zacharek A, Cui C, Zhang L, Li Q, Lu M, Zhang T, Liu A, Chen J. White matter damage and glymphatic dysfunction in a model of vascular dementia in rats with no prior vascular pathologies. *Neurobiol Aging*. 2017;50:96–106.
82. Back SA, Han BH, Luo NL, Chricton CA, Xanthoudakis S, Tam J, Arvin KL, Holtzman DM. Selective vulnerability of late oligodendrocyte progenitors to hypoxia-ischemia. *J Neurosci*. 2002;22:455–463.
83. Singh B, Singh V, Krishnan A, Koshy K, Martinez JA, Cheng C, Almquist C, Zochodne DW. Regeneration of diabetic axons is enhanced by selective knockdown of the PTEN gene. *Brain*. 2014;137:1051–1067.
84. Kodl CT, Seaquist ER. Cognitive dysfunction and diabetes mellitus. *Endocr Rev*. 2008;29:494–511.
85. Palacio S, McClure LA, Benavente OR, Bazan C, Pergola P, Hart RG. Lacunar strokes in patients with diabetes mellitus: risk factors, infarct location, and prognosis: the Secondary Prevention of Small Subcortical Strokes Study. *Stroke*. 2014;45:2689–2694.
86. Zhang J, Liu Z, Li Z, Wang Y, Chen Y, Li X, Chen K, Shu N, Zhang Z. Disrupted white matter network and cognitive decline in type 2 diabetes patients. *J Alzheimers Dis*. 2016;53:185–195.
87. Akisaki T, Sakurai T, Takata T, Umegaki H, Araki A, Mizuno S, Tanaka S, Ohashi Y, Iguchi A, Yokono K, Ito H. Cognitive dysfunction associates with white matter hyperintensities and subcortical atrophy on magnetic resonance imaging of the elderly diabetes mellitus Japanese elderly diabetes intervention trial (J-EDIT). *Diabetes Metab Res Rev*. 2006;22:376–384.
88. Linfante I, Cipolla MJ. Improving reperfusion therapies in the era of mechanical thrombectomy. *Transl Stroke Res*. 2016;7:294–302.
89. Pan J, Konstas A-A, Bateman B, Ortolano GA, Pile-Spellman J. Reperfusion injury following cerebral ischemia: pathophysiology, MR imaging, and potential therapies. *Neuroradiology*. 2007;49:93–102.
90. Ergul A, Elgebaly MM, Middlemore M-L, Li W, Elewa H, Switzer JA, Hall C, Kozak A, Fagan SC. Increased hemorrhagic transformation and altered infarct size and localization after experimental stroke in a rat model type 2 diabetes. *BMC Neurol*. 2007;7:33.
91. Ritter L, Davidson L, Henry M, Davis-Gorman G, Morrison H, Frye JB, Cohen Z, Chandler S, McDonagh P, Funk JL. Exaggerated neutrophil-mediated reperfusion injury after ischemic stroke in a rodent model of type 2 diabetes. *Microcirculation*. 2011;18:552–561.
92. Bas DF, Ozdemir AO, Colak E, Kebapci N. Higher insulin resistance level is associated with worse clinical response in acute ischemic stroke patients treated with intravenous thrombolysis. *Transl Stroke Res*. 2016;7:167–171.
93. Poppe AY, Majumdar SR, Jeerakathil T, Ghali W, Buchan AM, Hill MD. Admission hyperglycemia predicts a worse outcome in stroke patients treated with intravenous thrombolysis. *Diabetes Care*. 2009;32:617–622.
94. de Courten-Myers GM, Kleinholz M, Holm P, DeVoe G, Schmitt G, Wagner KR, Myers RE. Hemorrhagic infarct conversion in experimental stroke. *Ann Emerg Med*. 1992;21:120–126.
95. Ahnstedt H, Sweet J, Cruden P, Bishop N, Cipolla MJ. Effects of early post-ischemic reperfusion and tPA on cerebrovascular function and nitrosative stress in female rats. *Transl Stroke Res*. 2016;7:228–238.
96. Tarbell JM. Shear stress and the endothelial transport barrier. *Cardiovasc Res*. 2010;87:320–330.
97. Cunningham KS, Gotlieb AI. The role of shear stress in the pathogenesis of atherosclerosis. *Lab Invest*. 2004;85:9–23.
98. Jeong S-K, Lee J-Y, Rosenson RS. Association between ischemic stroke and vascular shear stress in the carotid artery. *J Clin Neurol*. 2014;10:133–139.
99. Gresele P, Guglielmini G, De Angelis M, Ciferri S, Ciofetta M, Falcinelli E, Lalli C, Ciabattoni G, Davì G, Bolli GB. Acute, short-term hyperglycemia enhances shear stress-induced platelet activation in patients with type II diabetes mellitus. *J Am Coll Cardiol*. 2003;41:1013–1020.
100. Geiss LS, Wang J, Cheng YJ, Thompson TJ, Barker L, Li Y, Albright AL, Gregg EW. Prevalence and incidence trends for diagnosed diabetes among adults aged 20 to 79 years, United States, 1980–2012. *JAMA*. 2014;312:1218–1226.
101. Kernan WN, Inzucchi SE, Viscoli CM, Brass LM, Bravata DM, Horwitz RI. Insulin resistance and risk for stroke. *Neurology*. 2002;59:809–815.
102. Ning R, Chopp M, Yan T, Zacharek A, Zhang C, Roberts C, Cui X, Lu M, Chen J. Tissue plasminogen activator treatment of stroke in type-1 diabetes rats. *Neuroscience*. 2012;222:326–332.
103. Fan X, Qiu J, Yu Z, Dai H, Singhal AB, Lo EH, Wang X. A rat model of studying tissue-type plasminogen activator thrombolysis in ischemic stroke with diabetes. *Stroke*. 2012;43:567–570.
104. Won SJ, Tang XN, Suh SW, Yenari MA, Swanson RA. Hyperglycemia promotes tissue plasminogen activator-induced hemorrhage by increasing superoxide production. *Ann Neurol*. 2011;70:583–590.
105. Caso V, Paciaroni M, Venti M, Palmerini F, Silvestrelli G, Milia P, Biagini S, Agnelli G. Determinants of outcome in patients eligible for thrombolysis for ischemic stroke. *Vasc Health Risk Manag*. 2007;3:749–754.
106. Rizk NN, Rafols JA, Dunbar JC. Cerebral ischemia-induced apoptosis and necrosis in normal and diabetic rats: effects of insulin and C-peptide. *Brain Res*. 2006;1096:204–212.
107. Liu H, Ou S, Xiao X, Zhu Y, Zhou S. Diabetes worsens ischemia-reperfusion brain injury in rats through GSK-3β. *Am J Med Sci*. 2015;350:204–211.
108. Cheng C, Daskalakis C. Association of adipokines with insulin resistance, microvascular dysfunction, and endothelial dysfunction in healthy young adults. *Mediators Inflamm*. 2015;2015:594039.
109. Kaji H. Adipose tissue-derived plasminogen activator inhibitor-1 function and regulation. *Compr Physiol*. 2016;6:1873–1896.
110. Chapin JC, Hajjar KA. Fibrinolysis and the control of blood coagulation. *Blood Rev*. 2015;29:17–24.
111. Trost S, Pratley RE, Sobel BE. Impaired fibrinolysis and risk for cardiovascular disease in the metabolic syndrome and type 2 diabetes. *Curr Diab Rep*. 2006;6:47–54.
112. Lijnen HR, Collen D. Impaired fibrinolysis and the risk for coronary heart disease. *Circulation*. 1996;94:2052–2054.
113. Demchuk AM, Morgenstern LB, Krieger DW, Linda Chi T, Hu W, Wein TH, Hardy RJ, Grotta JC, Buchan AM. Serum glucose level and diabetes predict tissue plasminogen activator-related intracerebral hemorrhage in acute ischemic stroke. *Stroke*. 1999;30:34–39.
114. Jotic A, Milicic T, Covickovic Sternic N, Kostic VS, Lalic K, Jeremic V, Mijajlovic M, Lukic L, Rajkovic N, Civcic M, Macesic M, Seferovic JP, Stanarcic J, Aleksic S, Lalic NM. Decreased insulin sensitivity and impaired fibrinolytic activity in type 2 diabetes patients and nondiabetics with ischemic stroke. *Int J Endocrinol*. 2015;2015:934791.
115. Wyseure T, Rubio M, Denorme F, Martinez de Lizarrondo S, Peeters M, Gils A, De Meyer SF, Vivien D, Declerck PJ. Innovative thrombolytic strategy using a heterodimer diabody against TAFI and PAI-1 in mouse models of thrombosis and stroke. *Blood*. 2015;125:1325–1332.
116. Sims NR, Muyderman H. Mitochondria, oxidative metabolism and cell death in stroke. *Biochem Biophys Acta*. 2010;1802:80–91.

117. Mishiro K, Imai T, Sugitani S, Kitashoji A, Suzuki Y, Takagi T, Chen H, Oumi Y, Tsuruma K, Shimazawa M, Hara H. Diabetes mellitus aggravates hemorrhagic transformation after ischemic stroke via mitochondrial defects leading to endothelial apoptosis. *PLoS One*. 2014;9:e103818.

118. Poulsen RC, Knowles HJ, Carr AJ, Hulley PA. Cell differentiation versus cell death: extracellular glucose is a key determinant of cell fate following oxidative stress exposure. *Cell Death Dis*. 2014;5:e1074.

119. Tang J, Li YJ, Li Q, Mu J, Yang DY, Xie P. Endogenous tissue plasminogen activator increases hemorrhagic transformation induced by heparin after ischemia reperfusion in rat brains. *Neurol Res*. 2010;32:541–546.

120. Doyle KP, Simon RP, Stenzel-Poore MP. Mechanisms of ischemic brain damage. *Neuropharmacology*. 2008;55:310–318.

121. di Penta A, Moreno B, Reix S, Fernandez-Diez B, Villanueva M, Errea O, Escala N, Vandenbroeck K, Comella JX, Villoslada P. Oxidative stress and proinflammatory cytokines contribute to demyelination and axonal damage in a cerebellar culture model of neuroinflammation. *PLoS One*. 2013;8:e54722.

122. Kim JY, Kawabori M, Yenari MA. Innate inflammatory responses in stroke: mechanisms and potential therapeutic targets. *Curr Med Chem*. 2014;21:2076–2097.

123. Whitney NP, Eidem TM, Peng H, Huang Y, Zheng JC. Inflammation mediates varying effects in neurogenesis: relevance to the pathogenesis of brain injury and neurodegenerative disorders. *J Neurochem*. 2009;108:1343–1359.

124. Pickup JC. Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes. *Diabetes Care*. 2004;27:813–823.

125. Wang C, Jiang J, Zhang X, Song L, Sun K, Xu R. Inhibiting HMGB1 reduces cerebral ischemia reperfusion injury in diabetic mice. *Inflammation*. 2016;39:1862–1870.

126. Maillard-Lefebvre H, Boulanger E, Daroux M, Gaxatte C, Hudson BI, Lambert M. Soluble receptor for advanced glycation end products: a new biomarker in diagnosis and prognosis of chronic inflammatory diseases. *Rheumatology*. 2009;48:1190–1196.

127. Qiu J, Xu J, Zheng Y, Wei Y, Zhu X, Lo EH, Moskowitz MA, Sims JR. High-mobility group box 1 promotes metalloproteinase-9 upregulation through toll-like receptor 4 after cerebral ischemia. *Stroke*. 2010;41:2077–2082.

128. Bright R, Mochly-Rosen D. The role of protein kinase C in cerebral ischemic and reperfusion injury. *Stroke*. 2005;36:2781–2790.

129. Lindsberg PJ, Roine RO. Hyperglycemia in acute stroke. *Stroke*. 2004;35:363–364.

130. Candelise L, Landi G, Orazio EN, Boccardi E. Prognostic significance of hyperglycemia in acute stroke. *Arch Neurol*. 1985;42:661–663.

131. Murros K, Fogelholm R, Kettunen S, Vuorela AL, Valve J. Blood glucose, glycosylated haemoglobin, and outcome of ischemic brain infarction. *J Neurol Sci*. 1992;111:59–64.

132. Anderson RE, Tan WK, Martin HS, Meyer FB. Effects of glucose and PaO_2 modulation on cortical intracellular acidosis, NADH redox state, and infarction in the ischemic penumbra. *Stroke*. 1999;30:160–170.

133. Nedergaard M, Gjedde A, Diemer NH. Hyperglycaemia protects against neuronal injury around experimental brain infarcts. *Neurol Res*. 1987;9:241–244.

134. Prado R, Ginsberg MD, Dietrich WD, Watson BD, Busto R. Hyperglycemia increases infarct size in collaterally perfused but not end-arterial vascular territories. *J Cereb Blood Flow Metab*. 1988;8:186–192.

135. Tsai M-J, Lin M-W, Huang Y-B, Kuo Y-M, Tsai Y-H. The influence of acute hyperglycemia in an animal model of lacunar stroke that is induced by artificial particle embolization. *Int J Med Sci*. 2016;13:347–356.

136. Robbins NM, Swanson RA. Opposing effects of glucose on stroke and reperfusion injury: acidosis, oxidative stress, and energy metabolism. *Stroke*. 2014;45:1881–1886.

137. Baird TA, Parsons MW, Phanh T, Butcher KS, Desmond PM, Tress BM, Colman PG, Chambers BR, Davis SM. Persistent poststroke hyperglycemia is independently associated with infarct expansion and worse clinical outcome. *Stroke*. 2003;34:2208–2214.

138. Cui C, Ye X, Chopp M, Venkat P, Zacharek A, Yan T, Ning R, Yu P, Cui G, Chen J. miR-145 regulates diabetes-bone marrow stromal cell-induced neurorestorative effects in diabetes stroke rats. *Stem Cells Transl Med*. 2016;5:1656–1667.

139. Long G, Wang F, Li H, Yin Z, Sandip C, Lou Y, Wang Y, Chen C, Wang DW. Circulating miR-30a, miR-126 and let-7b as biomarker for ischemic stroke in humans. *BMC Neurol*. 2013;13:178.

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