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REVIEW

Targeting brain microvascular endothelial cells: a therapeutic approach to neuroprotection against stroke

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Abstract

Brain microvascular endothelial cells form the interface between nervous tissue and circulating blood, and regulate central nervous system homeostasis. Brain microvascular endothelial cells differ from peripheral endothelial cells with regards expression of specific ion transporters and receptors, and contain fewer fenestrations and pinocytotic vesicles. Brain microvascular endothelial cells also synthesize several factors that influence blood vessel function. This review describes the morphological characteristics and functions of brain microvascular endothelial cells, and summarizes current knowledge regarding changes in brain microvascular endothelial cells during stroke progression and therapies. Future studies should focus on identifying mechanisms underlying such changes and developing possible neuroprotective therapeutic interventions.

Key Words: *nerve regeneration; blood-brain barrier; brain microvascular endothelial cells; cerebral infarction; subarachnoid hemorrhage; gap junction; endothelin; thromboxane A2; neural regeneration*

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Introduction

The blood-brain barrier (BBB) is the interface between blood components and neural tissue within the brain and spinal cord, which regulates homeostasis of the brain microenvironment for correct neuronal activity. The BBB is composed of four main cellular elements, namely, brain microvascular endothelial cells (BMECs), astrocyte end-feet, microglial cells, and pericytes. Brain function is maintained by BMECs *via* the BBB (Bleau et al., 2015). In contrast to the peripheral microvasculature, the brain microvasculature (with a blood vessel diameter < 20 μm) not only maintains blood supply, but also facilitates information transfer between neurons and glial cells (Wang et al., 2015). After years of research, BMECs are now recognized not only as a simple anatomical and physiological barrier, but also as a highly active metabolic system that synthesizes various materials to nourish nerves and regulate vasomotor function (Wang et al., 2015). Moreover, BMEC dysfunction can trigger brain tissue damage, such as stroke, traumatic brain injury, and neurodegenerative diseases, and these injuries exacerbate BMEC dysfunction *via* a feedback loop (Krueger et al., 2015; Liu et al., 2015). This review will focus on the recent progress made in determining the role of BMECs in stroke, as well as understanding the changes in cellular structure and function of BMECs after stoke, and emerging opportunities for potential therapeutic strategies.

Morphological Characterization of BMECs

The brain microvascular endothelium, a thin layer of connected and anchorage-dependent cells, which is influenced by chemical, physical, and mechanical stimuli, constitutes the interface between the bloodstream and the deformable solid vascular wall. At the microscopic level, endothelial cells (ECs) are flat with a central, elongated nucleus. ECs contain Weibel-Palade bodies, long rod-shaped storage and secretory organelles containing various factors such as von Willebrand factor and P-selectin. Electron microscopy studies of the BBB show that BMECs possess morphological and metabolic characteristics that are distinct from those found in peripheral tissue (Eliceiri et al., 2011).

In contrast to peripheral ECs, BMECs contain significantly fewer pinocytotic vesicles (which are sometimes completely absent), more mitochondria (indicative of important metabolic activity) (Villegas and Broadwell, 1993), and polarized expression of specific ion and peptide transporters (Brown et al., 2007). These carriers, which are located in the basement and apex membranes, tend to be highly stereospecific and participate in the selective transport of small molecules. Endothelial cytoplasm lacking fenestrations is typically present in peripheral tissue capillaries, and

under normal circumstances, non-specifically blocks blood-borne hydrophilic molecules and cells from entering neuronal tissue through the vessel wall (Engelhardt and Wolburg, 2004).

A thin layer called the glycocalyx, a hydrated mesh of negatively charged glycosaminoglycans, proteoglycans, glycoproteins, and glycolipids secreted by ECs, is located between the circulating blood and endothelium. The interface between BMECs is approximately 10–20 nm thick. Normally, ECs are connected at a junctional complex comprised of gap junctions, adherent junctions, and tight junctions, which mainly regulate information transfer (Lu et al., 2008) and mediate the so-called transcellular and paracellular pathways (Dejana et al., 2008). The BBB junction is well developed and contains numerous tight junctions. The two different structures form adhesion complexes between cells, generating a highly selective barrier against molecules through the vessel wall (Bazzoni, 2004; Coisne and Engelhardt, 2011; Dejana and Giampietro, 2012; D'Agnillo et al., 2013), and consequently subjecting junction proteins to insult during acute or chronic brain injury. Cytoplasmic proteins link membrane proteins to actin, the primary cytoskeletal protein for maintenance of structural and functional integrity of the vascular endothelium (Ballabh et al., 2004).

Each gap junction consists of two connexons, which correspond to contribution of each of two partner cells. Gap junction gating is regulated by connexin phosphorylation, and is much less frequent than tight junction gating in cerebrovascular ECs (Nielsen et al., 2012).

Adherent junctions are mainly formed by members of the cadherin family of adhesion proteins, which regulate actin shrinkage to alter connections between cells, and thereby control barrier permeability by activating cytosolic and cell membrane proteins (Harrison et al., 2011). ECs express relatively high levels of two cadherins: vascular endothelial (VE)-cadherin, a cell-type-specific cadherin, and neuronal cadherin (N-cadherin). These cadherins are also present in other cell types such as neural cells and smooth muscle cells (Bazzoni, 2004).

Tight junctions regulate the paracellular flux of hydrophilic molecules across the BBB, and play a crucial role in the BBB, the ultrastructure of which appears as sites of apparent fusion involving the outer leaflets of the plasma membrane of adjacent ECs (Ballabh et al., 2004). Tight junctions consist of three integral membrane proteins, namely, claudins (*e.g.*, claudin-5) (Pachter et al., 2003; D'Agnillo et al., 2013), occludin (Pachter et al., 2003), and junction adhesion molecules (JAM) (Pachter et al., 2003), as well as several cytoplasmic accessory proteins including ZO-1, cingulin, VE-cadherin, and PECAM-1. Claudins form tight junction strands and are believed to be the major transmembrane proteins of tight junctions. In immune replica electron microscopy, claudins exclusively localize to tight junction strands. Among the 24 members of the claudin superfamily, claudin-1 and claudin-5 are primarily expressed in the ECs of mammalian capillaries, particularly brain capillaries (Brown et al., 2007). Occludin, which was the first membrane protein identified within tight junctions, forms a homophilic dimer with other cells. Moreover, upon transfection into cells without tight junctions, occludin forms a tight junction-like structure. Normal expression and localization of other junctional proteins may compensate for occludin loss (Cummins, 2012). In a previous study, occludin-deficient mice exhibited complex gross and histological phenotypes including brain calcification. These findings indicate that occludin exerts more complex functions than simply serving as a building block in ECs and other tissues (Liu et al., 2012). The JAM family is comprised of three members: JAM-1, JAM-2, and JAM-3, which are expressed in rodent brain. Of these proteins, JAM-1 and JAM-3 are expressed in ECs (Yakubu et al., 2007). Nevertheless, understanding of the function of JAM is incomplete, and further studies are needed to determine the function of this family in the BBB. BMECs and tight junctions, as morphological components of the BBB, are closely packed and contain fewer plasma membrane vesicles and fenestrations than the analogous intervals in peripheral capillary ECs (**Table 1**).

Functions of BMECs

Forming the BBB and mediating transport

BMECs are a major cellular element of the BBB. Under physiological conditions, water-soluble substances and small molecules slowly pass through the BBB, whereas lipid-soluble molecules pass through more quickly. Studies have found that several compounds, such as glucose, amino acids, and exogenous drugs, enter the brain tissue though special transport proteins that are expressed in BMECs. These proteins include the transferrin receptor (Ohtsuki et al., 2013), insulin receptor (Begg, 2015), insulin-like growth factor receptor (Wang et al., 2013), and angiotensin receptor (Asashima et al., 2003). ECs also provide a metabolic barrier, expressing a number of enzymes that can degrade harmful and therapeutic molecules (Walter et al., 2015). Immune-mediated changes also specifically affect the structure and function of the BBB under normal physiological conditions and during cerebral pathological processes (Serres et al., 2009; Rochfort and Cummins, 2015).

BMECs in brain vascular contraction and diastole

The vascular endothelium is a highly active metabolic system that synthesizes various vascular regulatory factors to adjust the microcirculation of the cerebral tissue. Thus, integrity and function of EC structure is significant for vascular tone. Moreover, vasoconstrictors and vasodilators are balanced under physiological conditions (Gracia-Sancho et al., 2015).

Endothelin and nitric oxide

Research has now confirmed that vascular ECs synthesize and release endothelin-1 (ET-1) and nitric oxide (NO) (Yakubu and Leffler, 1999; Salani et al., 2000; Yakubu et al., 2007). Endothelin-1 is involved in blood vessel contraction and promotes EC proliferation, whereas NO relaxes vascular smooth muscle and inhibits vascular smooth muscle cell proliferation (Yakubu and Leffler, 1999; Salani et al., 2000; Yakubu et al., 2007). Under normal circumstances, ET-1 and NO are always in a dynamic balance to maintain function of vascular tone.

Disruption in this balance can trigger the occurrence of particular diseases, including stroke, which are the result of cerebral blood vessel dysfunction (Yakubu et al., 2007).

ET-1 was first identified, separated, and purified by Yanagisawa et al. (1988) and is an important vascular endothelial factor, with its synthesis controlled by intracellular Ca^{2+} signals, particularly after cerebral hemorrhage. The ET family contains three members, namely, ET-1 (expressed in ECs), ET-2 (expressed in kidneys and jejunum), and ET-3 (expressed in adrenal glands, jejunum, and kidneys) (Maguire and Davenport, 2015). The plasma concentration of ET is usually low, approximately 0.2–5 pg/mL, with a short half-life of 4–7 minutes. Brain tissue contains abundant ETs, particularly in the hypothalamus and striatum (Maguire and Davenport, 2015). Endothelin is synthesized through various signaling pathways in different ECs, and is also generated but not stored in BMECs (Stanimirovic et al., 1993; Yakubu and Leffler, 2002). In ECs, a certain amount of ET precursor is stored and will be immediately converted into ET under specific physiological and pathological conditions *e.g.*, cold, hypoxia, fluid shear stress, and production of vasoactive agents (such as adrenaline, angiotensin, vasopressin, thrombin, and endotoxin). Among these factors, hypoxia and shear forces are the main stimuli (Yakubu and Leffler, 1999; Maguire and Davenport, 2015). Endothelin decreases local brain blood flow *via* vasoconstriction, regulating thrombus formation though a platelet interaction, and thereby inducing cerebral infarction in several cases (Mamo et al., 2014). A previous study reported significantly increased ET-1 expression in animals with subarachnoid hemorrhage (SAH), hence ET-1 influences cerebral microcirculation following SAH (Lei et al., 2015).

NO is a simple, small biological radical and a multifunctional gaseous molecule, which is increasingly recognized to function as a signaling molecule (Fukumura et al., 2006; Forstermann and Sessa, 2012). Three nitric oxide synthase (NOS) genes with distinct tissue localization and properties have been identified. These genes are neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS), with the latter regulating endothelial function (Kim et al., 2011). Many investigators have detected NO production and eNOS activity using BMECs as a model. NO released into the vascular lumen functions as a potent inhibitor of platelet aggregation and vascular wall adhesion. Stimuli such as bradykinin, histamine, substance P, adenosine, and fluid shear stress upregulate NO expression and cause vasodilatation. Among these stimuli, fluid shear stress is the most established influencing factor. Furthermore, NO-mediated vasodilatation plays an important role in arteries rather than veins (Vanhoutte and Gao, 2013; Troelsen et al., 2015). Studies in mice deficient in eNOS ($eNOS^{-/-}$) show increased levels of amyloid-beta protein precursor (AβPP) and beta-site AβPP cleaving enzyme (BACE)-1 in the cerebral microvasculature and brain tissue (Austin et al., 2010, 2013).

Many physiological processes are promoted by NO and mostly synthesized though eNOS. These processes include smooth-muscle relaxation, neurotransmission (Mitsumori et al., 2011), inhibition of platelet aggregation, and adhesion to collagen and the vascular endothelium (Fukumura et al., 2006). Studies have also shown that eNOS inhibition reduces BBB disruption (Han et al., 2006; Beauchesne et al., 2009), and excess NO directly alters the BBB (Heo et al., 2005).

Thromboxane A2 (TXA2) and prostaglandin I2 (PGI2)

TXA2 and PGI2 are biologically active metabolites of arachidonic acid. When stably balanced, these factors are widely implicated in a range of physiological and pathological processes such as promoting platelet aggregation, vasoconstriction, and cancer proliferation (Smyth, 2010; Ekambaram et al., 2011). Additionally, TXA2 and PGI2 are associated with pathological processes such as microcirculatory dysfunction. TXA2 was one of the first prostaglandins to be identified from washed platelets (Hamberg et al., 1975), while PGI2 (or prostacyclin) was first reported by Needleman and Vane in 1976 (Needleman et al., 1976), and is the strongest known platelet aggregation inhibitor. PGI2 is expressed in many cell types, in particular ECs. A dynamic balance between TXA2 and PGI2 is required for vasomotor function. Dysfunction of the microvascular endothelium, especially during hypoxic ischemic injury, disrupts the TXA2/PGI2 balance, resulting in platelet activation and subsequent thrombogenesis (Smyth, 2010).

Involvement of BMECs in platelet activation and adhesion

BMECs are also involved in platelet activation. It is well known that von Willebrand factor is a multimeric plasma glycoprotein mainly synthesized in ECs, and a marker of acute and chronic EC activation (Perutelli and Molinari, 2007). With high shear stress, platelet adhesion at the site of vascular damage mainly functions as a bridge between the injured subendothelium and platelet receptors. Under physiological conditions, platelets circulate in every tissue in a resting state, and become activated by blood flow changes, vascular injury, or chemical stimuli. Although platelets do not normally physically interact with microvascular ECs, activated platelets bind to the wall of inflamed microvessels by directly attaching to BMECs or leucocytes, which are already adhered to the vessel wall (Waldner et al., 2012). Lack of platelet adhesion to healthy ECs has been partially attributed to inhibitory mechanisms involving NO, prostacyclin, and adenosine, which are normally generated by the vascular endothelium (Stokes and Granger, 2012). Although free radicals and thrombin are overexpressed, downregulated expression of endothelial NO and PGI2 aggravates platelet adhesion to BMECs, which in turn exacerbates microcirculatory dysfunction.

Protective effect of BMECs on brain tissue and neurons

Similar to many cells in other organs, BMECs exert a paracrine function (Li et al., 2009). The neurovascular unit, which is composed of ECs, astrocytes, and neurons, enables critical and metabolic tissue viability thresholds with cellular interactions that constitute the BBB. This unit also maintains normal physiological function of neurons and revascularization of injured vessels. For example, BMECs secrete

neurotrophins such as brain-derived neurotrophic factor (BDNF), insulin-like growth factor 1 (IGF-1), and vascular endothelial growth factor (VEGF) (Shimizu et al., 2012).

BDNF is involved in ensuring synaptic plasticity and neuronal survival (Suliman et al., 2013), and is synthesized in the central nervous system at low levels during development and higher levels during the postnatal period. However, in addition, BDNF is also synthesized and secreted by BMECs (Leventhal et al., 1999; Bayas et al., 2002; Kim et al., 2004). A previous study showed that BDNF secretion in circulating blood increases during early acute ischemia (up to 3 hours). After 48 hours, another peak is produced by activation of the target of rapamycin complex 1 (TORC1)/cAMP response element-binding (CREB)/BDNF signaling pathway (Gallo and Iadecola, 2011).

IGF-1 is a circulating hormone generated not only in the liver, but also locally in many cell types (such as neurons, glia, and cerebral microvascular ECs) (Wang et al., 2013), and is essential for nervous system development, hippocampal neurogenesis, and neurotransmission. In animal models of stroke, IGF-1 exerts neuroprotective effects, and high serum IGF-1 levels immediately after the onset of ischemic stroke are associated with improved neurological recovery and functional outcome. Hence, the evolution of cerebral infarction is affected by endogenous IGF-1 levels (De Smedt et al., 2011).

VEGF is a neuronal and glial trophic factor (Fusco et al., 2014) that is synthesized by several cell types including ECs, macrophages, activated platelets, T lymphocytes, smooth muscle cells, kidney cells, keratinocytes, osteoblasts, cancer cells, and brain cells (*e.g.*, astrocytes and neuronal stem cells). Furthermore, VEGF promotes proliferation and survival of ECs and stimulates NO-dependent vasodilation. Moreover, VEGF influences vasculature formation and increases vascular permeability. In the brain, VEGF participates in angiogenesis during embryonic and postnatal development (Nowacka and Obuchowicz, 2012).

In addition, BMECs also secrete matrix metalloproteinases (MMPs), which are key components in proteolytic BBB disruption during ischemic stroke, and contribute to vascular edema, hemorrhagic transformation, and leukocyte infiltration (Del Zoppo, 2010; Reuter et al., 2013) (**Figure 1**).

Structural and functional changes in BMECs during stroke

Stroke is the second leading cause of death worldwide and the leading cause of acquired disability in adults in most regions. Stroke can be classified as ischemic or hemorrhagic, with approximately 80% of stroke cases being ischemic (O'Donnell et al., 2010; Wang, 2011). In the 17th century, Johann Jacob Wepfer defined stroke as a vascular problem. The history of research on stroke pathophysiology reflects a shift in focus from a purely vascular conception to the involvement of a complex interplay of biochemical and molecular mechanisms involving all brain cell types, particularly BMECs, in salvage or demise of tissue affected by stroke. In addition, the neurovascular unit plays a crucial role in stroke development. Under physiological conditions, integrity of the structure and function of BMECs maintain the BBB, but change with stroke progression. Damaged microvascular ECs trigger a series of cerebrovascular injuries, which in turn exacerbate BMEC injury. However, the precise relationship between BMECs and brain injury remains unclear. Recent studies have shown that patients with BMEC dysfunction, tight junction degradation, and disrupted cytokine secretion and electrolyte balance are vulnerable to stroke (Lapi and Colantuoni, 2015; Rochfort et al., 2015) (**Table 2**).

BMECs in ischemic stroke

During ischemic stroke, oxygen and nutrient deprivation activates proteases, resulting in degradation of tight junction proteins in BMECs and increased BBB permeability (Hamann et al., 2003). An *in vitro* study found that human BMECs participate in MMP-mediated BBB breakdown during ischemic stroke by creating a proinflammatory state with enhanced MMP-2 production and attenuated tissue inhibitor of metalloproteinases 1 (TIMP-1) release, an endogenous MMP inhibitor (O'Donnell et al., 2010).

Ultrastructural observations show that capillary tight junctions can be classified into various stages of degradation, which indicates that a large volume of blood-brain fluid and components (MD > 360 kDa) enters the brain tissue *via* capillaries. However, under physiological conditions, this large volume of blood-brain fluid and blood fluid components cannot pass through the BBB (Hamann et al., 2003). A previous microstructural examination revealed that BMECs show endoplasmic reticulum swelling and formation of numerous large cytoplasmic vacuoles, while mitochondria in the cytoplasm of most cells have disrupted cristae (Garbuzova-Davis et al., 2013). Microvilli fragments were observed to freely float in the capillary lumen, in addition to evident cristae disruption in the mitochondria of ECs. Large autophagosomes were detected in almost all ECs, with some autophagosomes extending from the lumen to the basal lamina in attenuated cell portions (Garbuzova-Davis et al., 2013). Osmiophilic debris in areas of microvessel damage indicates ruptured autophagosomes. With increasing time, nuclear chromatin pyknosis and BMEC swelling also worsened. After 1–2 weeks, some swollen BMECs burst into and narrowed the vessel lumen. In addition, ischemic stroke induced other EC changes, such as loss of matrix ligands, EC and astrocyte integrin receptors, and expression of members of several matrix-degrading protease families. This further leads to increased BBB permeability (Del Zoppo and Mabuchi, 2003). However, several ultrastructural studies have shown that tight junction changes are rarely observed 5–25 hours after ischemic stroke (Krueger et al., 2013). Along with ultrastructural changes, microvascular endothelium functional changes are also found.

ET-1 is a potent vasoconstrictor that induces direct angiogenic effects on ECs (Spinella et al., 2010). Piamsomboon showed that enhanced ET-1 expression in the serum and cerebrospinal fluid of patients with cerebral infarction is ameliorated using prostaglandin E1 (PGE1), which protects against brain ischemia-reperfusion injury (Piamsomboon et al., 2007). Expression of ET-1 is upregulated during the acute period of ischemic stroke. Moreover, no detectable

Table 1 Connected junctional complex of brain microvascular endothelial cells

Name	Family	Formation	Formation members
Junctional complex	Gap junctions (GJs)	Each gap junction is made up of two connexons	None
	Adherent junctions (AJs)	Cadherin family of adhesion proteins	Vascular endothelial (VE)-cadherin
			Neuronal (N)-cadherin
	Tight junctions (TJs)	Integral membrane proteins	Claudin family: claudin-1 and claudin-5
			Occludin family
			Junction adhesion molecules (JAM) family: JAM-1 and JAM-3
		Cytoplasmic accessory proteins	–
	Transcellular and paracelluar pathways	–	–

Table 2 Changes in structure and function of brain microvascular endothelial cells during stroke

		Stroke	
Changes	Components	Ischemic stroke	Subarachnoid hemorrhage
Structure	Tight junctions	Tight junction degradation	–
	Ultrastructure observation	Endoplasmic reticulum swelling	Edema
		Numerous large vacuoles formation	Cell nucleus pycnosis of brain microvascular endothelial cells
		Fragments of microvilli floating free in the capillary lumen	Degranulation of rough endoplasmic reticulum
		Disruption of the cristae in the mitochondria	Disruption of the cristae in the mitochondria
		Autophagosomes	Leukocyte receptors and adhesion molecule increased
Functions	Endothelin-1	↑ (No relationship)	↑ (Destructive)
	Nitric oxide	↑ (Protective and destructive)	↓ (Destructive)
	Thromboxane A2	↑ (Destructive)	↑ (Destructive)
	Prostaglandin I2	↑ (Protective)	↓ (Destructive)
	Platelet activation	↑ (Destructive)	↑ (Destructive)
	Brain derived neurotrophic factor	↑ (Protective)	↑ (Protective)

↑ : Increased; ↓ : decreased.

difference is observed between stroke patients and normal subjects after 7 days. This phenomenon can be explained by locally increased ET-1 production from damaged ECs within infarcted tissue (Sapira et al., 2010). Other studies have shown that plasma ET-1 levels are not related to cerebral infarction size, location, degree of clinical neurological defects, or prognosis (Sapira et al., 2010; Hung et al., 2015).

NO is synthesized by three distinct forms of NOS, each of which behaves differently under ischemic situations. In the early ischemic period (up to 2 hours), NO synthesized by microvascular ECs triggers vasodilation, exerts a protective effect, inhibits platelet aggregation, and increases blood flow to affected brain regions. Studies have shown that eNOS inhibition ameliorates BBB disruption (Han et al., 2006; Beauchesne et al., 2009) and excess NO directly alters the BBB (Heo et al., 2005).

BDNF is closely related to cerebral ischemic or anoxic injury (Cui et al., 2010; Yang et al., 2015). The BDNF cascade activates Raf-1 MEK1/2/ERK1/2 protein kinases, inhibiting cell apoptosis and exerting a neuroprotective effect (Sung et al., 2012). Furthermore, BDNF confers protection against neuronal loss caused by cerebral ischemia (Mattson et al., 2004) and reduces infarct volume in different stroke models (Shi et al., 2009). Expression of BDNF is upregulated after SAH (Mattson et al., 2004; Shi et al., 2009) and ameliorated after ischemic brain injury in a mouse model of focal cerebral ischemia. This process may be a protective response following ischemic injury.

BMECs in hemorrhagic stroke

Intracerebral hemorrhage, a fatal stroke subtype, currently has no effective treatment option. Even if patients survive the initial attack, the growing hematoma triggers a series of life threatening events leading to accumulation of cerebral edema, progression of neurobehavioral deficits, and possible death (Strbian et al., 2008; Fiorella et al., 2015). The toxic effect of extravasated blood leads to secondary damage after the initial injury, and causes death of neighboring cells due to free radical generation and oxidative damage (Nakamura et al., 2005). Furthermore, BMEC dysfunction may lead to BBB disruption, which is a hallmark of intracerebral hemorrhage-induced brain injury. Such disruption contributes to edema formation, leukocyte influx, and entry of potentially neuroactive agents into the perihematomal brain, all of which contribute to brain injury (Leclerc et al., 2015).

In cerebral vasospasm, apoptosis of ECs and neurons is a secondary consequence of SAH. Cerebral vasospasm can be divided into two phases (Weir et al., 1978): acute (3–4 hours after SAH) and chronic (3–4 days after SAH). Continuous artery stenosis is a characteristic of the chronic phase and significantly decreases the survival rate of SAH patients (Chaichana et al., 2010). Cerebrospinal fluid levels of ET-1 increase during severe neuronal damage, regardless of whether the damage is due to vasospasm or a primary hemorrhagic event. In addition, cerebrospinal fluid levels of ET-1 correlate with neurological deterioration but are not predictive of vasospasm (Mascia et al., 2001). Animal studies show that NO-generating agents such as nitrite, reduce cerebral vasospasm (CVS) and improve prognosis in animal models of cerebral hemorrhage. Nevertheless, no relevant human studies are currently available (Fathi et al., 2011; Jung et al., 2011).

Microvessel injury is a serious secondary event of SAH, and potential main mechanisms include inflammation, oxidative stress injury, platelet activation, long-term vasoconstriction, and EC apoptosis (Tso and Macdonald, 2014). Intravascular activation of blood cells leads to scattered microvessel plugging, increased vascular permeability, edema formation, and cytotoxic action of blood cell-released agents on the underlying tissue, all of which may be involved in SAH (Akopov et al., 1996; Ding et al., 2014). Microvasculature injury is more serious and occurs faster than cerebral damage (Cao et al., 2015). In addition, EC apoptosis occurs 24 hours after SAH (Friedrich et al., 2012), with enhanced expression of p53-upregulated modulator of apoptosis (PUMA), BAX, BAK, GRP78, and DRP1 in microvascular hippocampal ECs. This indicates that PUMA-evoked EC apoptosis significantly affects BBB disruption following SAH, and may be mediated through the endoplasmic reticulum (Yan et al., 2011). However, one study suggested that changes in the gaps between BMECs are not obvious (Scharbrodt et al., 2009). Furthermore, leukocyte receptor expression on microvascular ECs, and cytokine and adhesion molecules increase in the blood and cerebrospinal fluid (Bavbek et al., 1998; Polin et al., 1998).

Plasma thrombin expression increases after cerebral hemorrhage (Yan et al., 2013). Although the pathogenesis of chronic CVS remains uncertain, the underlying mechanism may be inflammation (after the initial hemorrhage), specifically from the interaction between leukocytes and cell adhesion molecules in the vascular endothelium (Li et al., 2015; Shao et al., 2015). After SAH, leukocyte migration causes damage to BMECs that is exacerbated by platelet-activating factor (Akopov et al., 1995).

Expression of BDNF after SAH also increases. Genetically influenced variation in BDNF function is associated with recovery from SAH, thus targeting BDNF signaling may facilitate recovery from brain injury (Siironen et al., 2007).

BMECs in neuroprotective stoke therapies

Therapeutic strategies have been developed to modulate vasomotion, thrombosis, and protection of microvascular ECs against cerebral stroke. Treatment with PUMA siRNA significantly reduces mortality, cerebral edema, neurobehavioral deficits, and BBB disruption following SAH injury (Yan et al., 2011). In addition, pifithrin-alpha, a p53 inhibitor, protects cerebral vessels from vasospasm development and improves neurological outcome by decreasing EC apoptosis and alleviating CVS, as well as reducing mortality rate by suppressing p53-induced apoptosis in cerebral vessel ECs (Yan et al., 2008). Some researchers have found that the protease-activated receptor 1 (PAR-1) antagonist, SCH79797, preserves microvascular integrity and provides neurobehavioral protection, which is partly mediated by suppression of VE-cadherin endocytosis induced by c-Src-dependent PAK1 activation (Lee and Hamilton, 2013; Manaenko et al., 2013; Yan et al., 2013).

Oleandrin is the principal cardiac glycoside component of PBI-05204 (a supercritical CO_2 extract of *Nerium oleander*), which increases BDNF expression at the protein and transcriptional levels, indicating that PBI-05204 can provide neuroprotection against ischemic stroke (Van Kanegan et al., 2014). Increasing BDNF expression improves prognosis after stroke (Zhang et al., 2013; El-Tamawy et al., 2014), whereas several strategies for decreasing BDNF expression lead to a poorer prognosis (O'Keefe et al., 2014).

Aside from BMEC dysfunction, disruption at junctions between BMECs can induce stroke. Regulation of tight and adherent junctions of the BBB have been a popular therapeutic target. EC injury and cerebrovascular accident exhibit a cause and effect relationship, with the resulting mortality decreased through detection of EC apoptosis, and *vice versa* (Blanchette and Daneman, 2015). A recent study found that BMEC transplantation improves locomotor function, enhances remyelination of the injured internal capsule, and suppresses inflammatory responses in infarcted white matter (Puentes et al., 2012). In addition, the inflammatory response 2 weeks after BMEC transplantation is repressed compared with vehicle ischemia without BMECs, as evidenced by the smaller number of microglial cells positively activated by the microglia/macrophage antigen (ED-1) than that of bovine serum albumin-injected brains or meningeal cell-transplanted brains (Puentes et al., 2012). A relationship between BMECs and protection of white matter ischemia injury is rarely reported. Elucidation of the molecular mechanisms of BMECs

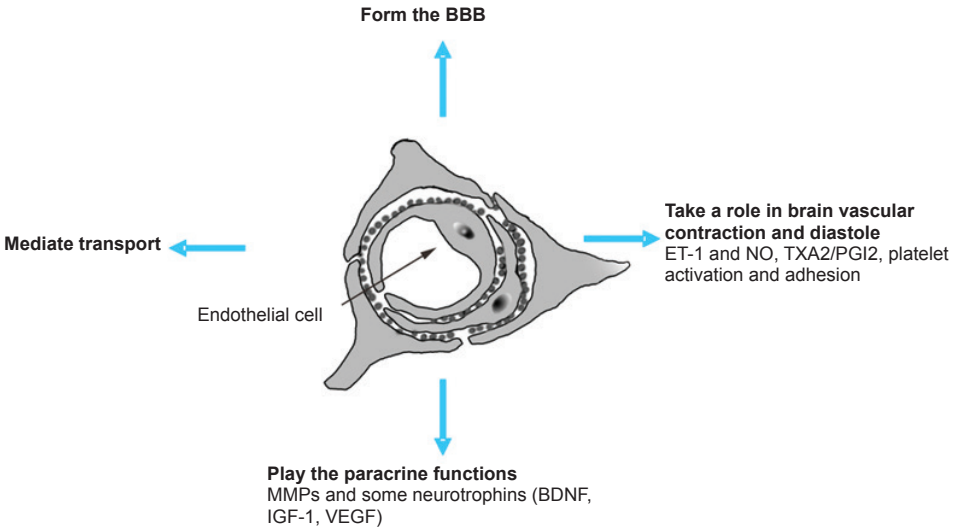

Figure 1 Function of brain microvascular endothelial cells.
Brain microvascular endothelial cells (BMECs) have many important functions such as BBB formation, mediating transport, brain vascular contraction and diastole, and paracrine function. BBB: Blood-brain barrier; ET-1: endothelin-1; NO: nitric oxide; TXA2: thromboxane A2; PGI2: prostaglandin I2; BDNF: brain-derived neurotrophic factor; IGF-1: insulin-like growth factor-1; VEGF: vascular endothelial growth factor.

in stroke may provide important insight into the development of effective therapies for white matter ischemia.

Under the stimulus of physiological or pathological factors, bone marrow-derived endothelial progenitor cells (EPCs) migrate to peripheral blood, where they participate in repair of damaged blood vessels and angiogenesis in ischemic tissue (Palladino et al., 2012). Recent studies on EPCs provide novel and promising potential therapies for the treatment of ischemic stroke and improvement of prognosis (Zengin et al., 2006; Li et al., 2015). After ischemia, EPCs migrate from the bone marrow to repair damaged sites either by direct incorporation of EPCs or repopulation of mature ECs. Following acute ischemic stroke, circulating EPCs are strikingly increased in order to execute their repair function on cerebrovascular trauma. As shown in an acute ischemic stroke study, circulating EPC counts peak at day 7, while statin pretreatment increased EPC levels. In patients with large-artery atherosclerosis and small-vessel disease subtypes, high EPC counts are related to improved outcome at 3 months (Martí-Fàbregas et al., 2013; Tsai et al., 2014).

Conclusions

Over the past decades, many clinical trials of neuroprotective agents have been performed with various single drugs or combinations of agents (Pandya et al., 2011). Nevertheless, therapeutic choices for stroke patients remain limited, with varied final outcomes of chronic disability, depending on the size and location of the infarct area. Although several functional recovery techniques have been achieved using various treatments, further studies are required to identify more effective treatments. Traditional methods are aimed at improving oxygen and nutrition supply to the ischemic area through interventions such as vasomotor adjustment, thrombosis prevention, and dynamic adjustment of brain blood flow after stroke (Lansberg et al., 2012).

Many studies have provided evidence to indicate that structural damage and BMEC dysfunction are involved in stroke. As discussed, BMECs act differently in different kinds of stroke and in different phases. Development of stroke involves changes in microvascular endothelial and related cytokine levels. Some of these changes, such as disturbed TXA2/PGI2 balance, platelet activation, and decreased BDNF, are subsequent events of cerebral infarction or cerebral hemorrhage, and under pathological conditions they may exacerbate cerebral infarction and reduce survival. Considering that the processes during stroke are extremely complex, therapeutic targets should focus on preventive protection of the integrity of BMEC structure and function. Although more research is required to highlight the role of BMECs in stroke pathogenesis, additional strategies that target newly identified signaling pathways or molecules may offer a promising therapeutic approach to stroke.

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