Blood–Brain Barrier Breakdown in Acute and Chronic Cerebrovascular Disease

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Abstract—Disruptions of the blood–brain barrier (BBB) and edema formation both play key roles in the development of neurological dysfunction in acute and chronic cerebral ischemia. Animal studies have revealed the molecular cascades that are initiated with hypoxia/ischemia in the cells forming the neurovascular unit and that contribute to cell death. Matrix metalloproteinases cause reversible degradation of tight junction proteins early after the onset of ischemia, and a delayed secondary opening during a neuroinflammatory response occurring from 24 to 72 hours after. Cyclooxygenases are important in the delayed opening as the neuroinflammatory response progresses. An early opening of the BBB within the 3-hour therapeutic window for tissue-type plasminogen activator can allow it to enter the brain and increase the risk of hemorrhage. Chronic hypoxic hypoperfusion opens the BBB, which contributes to the cognitive changes seen with lacunar strokes and white matter injury in subcortical ischemic vascular disease. This review will describe the molecular and cellular events associated with BBB disruption and potential therapies directed toward restoring the integrity of the neurovascular unit. (Stroke. 2011;42:3323-3328.)

Key Words: blood–brain barrier ▪ brain ischemia ▪ endothelium ▪ ischemia ▪ matrix proteins ▪ neuroprotection ▪ vascular cognitive impairment ▪ white matter disease

Molecular biology methods and advances in magnetic resonance imaging (MRI) have provided novel insights into the cerebral vasculature in acute and chronic stroke. Protection of the neuronal microenvironment is provided by the neuro(glio)vascular unit (NVU) that includes endothelial cells, astrocytes, pericytes, neurons, and extracellular matrix around the vessels.1 Tight junction proteins form the first defense in the endothelial barrier disruption that leads to vasogenic edema and cell death. In stroke, the ischemic injury induces a molecular cascade that culminates in the formation of toxic proteases and free radicals, which participate in damage to the tissue and the removal of dead cells. However, the same molecules that are damaging in the early stages of the injury perform essential functions in the recovery phase, drastically complicating the therapeutic use of these agents. The best-studied of these dual functioning molecules are the matrix metalloproteinases (MMP), cyclooxygenases (COX), as well as the free radicals, nitric oxide and reactive oxygen species.

Biology of Tight Junctions and the NVU
Tight junction proteins form the initial barrier at the endothelial cells between blood and brain cells. Major tight junction proteins are occludin and claudins close to the blood, and junctional adhesion molecules deeper in the endothelial cell clefts.2 Surrounding the abluminal surface of the endothelial cell is a basal lamina composed mainly of Type-IV collagen, fibronectin, heparan sulfate, and laminin; laminin functions as a charge and molecular weight barrier and interacts in complex ways with integrins to regulate permeability and cellular transport across the blood–brain barrier (BBB).3 Embedded in the basal lamina are the pericytes, which are hybrid cells with both macrophage and smooth muscle properties.4 Astrocytes are generally thought to be an essential cell for tight junction formation. However, a recent study in embryos challenges this concept by showing that BBB qualities of the capillary form when endothelial cells invade the central nervous system and pericytes are recruited to the developing vessels; this occurs more than a week before astrocyte generation, suggesting that pericytes are critical for tight junction formation.5 Another interesting study of pericytes showed that they decrease with age, paralleling an increase in BBB permeability.6

Disruption of the BBB in Ischemia/Hypoxia and Hemorrhage
Proteases, which are the final common pathway for disruption of the NVU, are normally present in a latent form. Some proteases are constitutively expressed and participate in normal processes. This includes 72-kDa gelatinase A (MMP-
2), which is normally found in cerebrospinal fluid and in astrocytes. Activation of the latent MMP-2 to the 62- to 64-kDa forms requires the formation of a trimolecular complex with MMP-2, tissue inhibitor of metalloproteinases-2 (TIMP-2), and membrane-type 1 MMP, which confines activity of MMP-2 to the vicinity of the membrane.

Another group of proteases are induced during an injury. The major inducible MMPs are stromelysin-1 (MMP-3) and 92-kDa gelatinase B (MMP-9). When these are released, they are unrestrained and act on multiple substrates in the extracellular matrix (Figure 1). Cytokines induce the expression of MMP-3 and MMP-9 through the action of nuclear-factor-κB and the activator protein-1 gene transcription sites. Another source of MMPs is through the influx of white blood cells, such as neutrophils, which can release activated 84-kDa MMP-9. In reperfusion injury, proteases participate in the biphasic opening of the BBB. An initial reversible phase related to activation of latent MMP-2 precedes a later phase at 24 to 48 hours associated with the induction of MMP-3 and MMP-9 (Figure 2).

COX is another important family of inflammatory enzymes. COX-1 is a constitutive enzyme, whereas COX-2 is inducible and contributes to BBB damage as part of a secondary inflammatory response from 24 to 72 hours after the initial insult. Both MMP and COX-2 inhibitors protect the BBB after an ischemic injury; MMP inhibitors act during the early phase, and COX-2 inhibitors block the secondary opening of the BBB, suggesting that a combination of both agents may be able to control better the disruption of the BBB.

Tissue-type plasminogen activator (tPA) breaks fibrin clots and restores blood flow. In a small percentage of patients, tPA leads to a life-threatening hemorrhage. Because the BBB is disrupted early in the injury cascade, a possible mechanism of bleeding is that tPA crosses the BBB. When tPA enters the brain rather than remaining in circulation, it induces cytokines and activates MMP-9.8 MMP inhibitors prevent early opening of the BBB and can reduce risk of hemorrhage and death when tPA is given in stroke.9 Low-density lipoprotein receptor-related protein is a serine protease that is a member of the low-density lipoprotein receptor gene family. Low-density lipoprotein receptor-related protein binds tPA and participates in the action of tPA.10,11

Aquaporins are pore-forming molecules that facilitate the passage of water molecules across the BBB. Deletion of AQP4 reduces edema in models in which cytotoxic edema is the pathophysiological mechanism. However, in conditions under which vasogenic edema is significant, AQP4 deletion exacerbates brain edema, because AQP4 functions as a passive pore; it allows water to follow pressure gradients to remove extracellular fluid and resolve vasogenic edema.12

Oxidative stress damages endothelial cells of the BBB and contributes to vasogenic edema. The superoxide radical \( \cdot O_2^– \) has been identified as the primary reactive oxygen species involved in increased vascular permeability and
edema formation in global and focal cerebral ischemia, cold brain injury, and brain tumors. Scavenging $O_2^-$ radicals using recombinant superoxide dismutase or poly-ethylene glycol-superoxide dismutase reduces ischemia-induced BBB injury and vasogenic edema. Superoxide dismutase-overexpressing mice also have reduced activation of MMP-9 by reactive oxygen species, which may be involved in early BBB disruption and progressive striatal damage induced by the mitochondrial excitotoxin, 3-nitropropionic acid.13

Normobaric hyperoxia in the early stages of stroke reduces disruption of the BBB in reperfusion injury.14 The increased oxygen acts on cells in the penumbra. When hyperoxia is used with tPA, there is reduction in hemorrhagic complications from tPA, probably because of the protection of the BBB. Rats treated with combined normobaric hyperoxia and tPA showed significantly reduced tPA-associated mortality, brain edema, hemorrhage, and MMP-9 induction compared with tPA alone.15

**Blood–Brain Barrier Studies in Humans**

In the early stages of an infarct in humans, blood vessels have increased permeability, which can be seen in the meninges over the area of the stroke. Gadolinium (Gd) enhancement on fluid-attenuated inversion recovery (FLAIR) images obtained several hours after injection showed disruption of the BBB, which appeared as enhancement in sulci over the infarct area. This was termed hyperintense acute reperfusion marker, and was found in one third of ischemic stroke patients; those with the sign had a higher risk of hemorrhagic transformation and worse clinical outcome compared with those who did not (Figure 3).16

Growth of intracerebral hemorrhage occurs in the first 24 hours.17 Treatment with Factor VII, which promotes clotting, in an early study reduced the growth, but in a second study failed to show an effect, and there is no proven treatment to reduce lesion growth in intracranial hemorrhage.18 The extent of hemorrhagic transformation after stroke correlated with MMP-9 elevation in the blood.19

**Blood–Brain Barrier Dysfunction in Chronic Cerebrovascular Disease**

BBB abnormalities have been described in patients with small-vessel disease secondary to hypertension and diabetes. Cerebrospinal fluid studies have documented increased albumin, which is an indicator of a disrupted BBB.20 Dynamic contrast-enhanced MRI provides a quantitative measurement of permeability.21 Patients with large white matter hyperintensities and symptoms suggestive of subcortical ischemic vascular disease had increased BBB permeability as measured by dynamic contrast-enhanced MRI, which paralleled changes in cerebrospinal fluid albumin index (Figure 4).22

Blood vessels undergo profound changes with aging. Tortuosity of the vessels and thickening of the vessel walls is found at autopsy in elderly patients, which results in reduced vascular reactivity in the regions of the white matter with hyperintensities on MRI.23 Hypertension, diabetes, and hyperlipidemia are the major factors besides age that cause changes in the blood vessels responsible for the impairments in cerebral blood flow and oxygenation, along with increase in permeability.24,25 The vulnerability of white matter is caused by multiple factors because it is the end of arterial circulation to the brain.26 Large epidemiological studies utilizing MRI and cognitive testing show a high incidence of changes in white matter after age 65 years associated with cognitive decline.27 There is a gradual growth in white matter lesions over time.28

Autopsy studies show the presence of hypoxia inducible factor-1$\alpha$ in affected white matter supporting a role of hypoxia in the process that leads to death of the oligodendrocytes and extensive gliosis.29 Several etiologies are proposed to explain changes in white matter. Silent strokes are suspected in many cases, and may be the initiating event, particularly when hypertension is damaging blood vessels.30 An alternative mechanism is disruption of the NVU with hypoxic edema. Support of a disturbed BBB comes from autopsy studies of patients withBinswanger’s disease that show the presence of serum proteins in the brain.31 A possible cause of BBB damage is the presence of MMPs in white matter at autopsy in patients with vascular dementia.32 Reactive astrocytes that are positive for glial fibrillary acidic protein are immunoreactive for MMP-2, whereas microglia/macrophages stain for MMP-3.

Mechanisms to explain the inflammatory process have been revealed by animal studies. Rats with hypoxic hypoperfusion induced by bilateral carotid artery occlusion have
increased MMP-2 in white matter and have chronic gliosis; MMP-2 knockout mice had less disruption of the BBB and damage to white matter.33

Translational Aspects of Blood–Brain Barrier Disruption in Stroke

MMP inhibitors (MMPI) block the early opening of the BBB, and COX-2 inhibitors are effective in the delayed phase. Several MMPIs have been tested and shown to have efficacy in animals, including BB-94, BB-1101, and GM6001 along with selective COX-2 inhibitors.34,35 Joint problems occurred when MMPIs were given chronically to cancer patients,36 but short-term use of MMPIs may avoid side-effects.37

Minocycline is an MMPI with multiple actions, including anti-inflammatory effects, and is well-tolerated in low doses over long periods of time.38 Treatment with minocycline benefits patients with multiple sclerosis, most likely through protection of the BBB.39 Another potential treatment is the use of COX-2 inhibitors, which block the secondary opening of the BBB in experimental stroke. Concern about cardiac side effects, which were seen with long-term use of these agents in arthritis, is less likely to be present in the short-term use.

Contrary to their deleterious actions in the early stages of an acute stroke, MMPs may aid in the recovery process. Poststroke angiogenesis is the key step for recovery after ischemia and provides critical neurovascular substrates for neuronal remodeling after stroke.40 In the process of angiogenesis, loss of vascular integrity and degradation of cell matrix are crucial initiating steps. MMPs degrade the extracellular matrix and prepare the stage for growth factors and guidance molecules. MMPs can also cleave many signaling molecules, such as vascular endothelial growth factor, which induces the proliferation of endothe-
molecules targeted for treatment. Many of those molecules that participate in the death of cells in early stages of injury also play a critical role in the recovery period. An example is that the benefit derived from MMP1 treatment in the early stages of injury may be lost if the same enzymes are blocked later in angiogenesis and neurogenesis. Understanding timing of the expression of each of the agents to be blocked and their actions at each point of the injury cycle is necessary in planning the use of inhibitors. In the future, as multiple cascades are better understood, treatments will be tailored to start when the damaging effects of that agent are maximal and to be stopped as the beneficial effects begin.

In conclusion, we have described some of the major advances in understanding the function of the NVU at the molecular level. Unraveling the proteins that comprise tight junctions provided tools to observe the effects of proteases, such as MMPs, on tight junctions following acute stroke. Agents that block BBB disruption can protect the brain from adverse effects of tPA, extending the therapeutic window. The combined effect of agents that act early, including MMPs and those that protect delayed opening of the BBB, such as COX-2 inhibitors, need to be tested. Recent studies of chronic effects of hyperfusion in humans and animals demonstrate a role for MMPs in both disruption of the BBB and the breakdown of myelin, which may contribute to the death of oligodendrocytes. Defining the molecular mechanisms underlying damage to the vasculature provides important information on which to base additional trials of novel therapies to protect the BBB.

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Disclosures
None.

References


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