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Triggers and Mediators of Hemorrhagic Transformation in Cerebral Ischemia

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Abstract

Intracerebral hemorrhagic transformation is a multifactorial phenomenon in which ischemic brain tissue converts into a hemorrhagic lesion with blood-vessel leakage, extravasation, and further brain injury. It has been estimated that up to 30–40% of all ischemic strokes undergo spontaneous hemorrhagic transformation, and this phenomenon may become even more prevalent with the increasing use of thrombolytic stroke therapy. An emerging conceptual model suggests that the loss of microvascular integrity and disruption of neurovascular homeostasis connects the experimental findings of blood-cell extravasation to brain injury after hemorrhage. In this short article, we examine mechanisms related to reperfusion injury and oxidative stress, leukocyte infiltration, vascular activation, and dysregulated extracellular proteolysis as potential triggers of hemorrhagic transformation. Perturbations in cell-cell and cell-matrix signaling within the hypothesized neurovascular unit may ultimately lead to neuroinflammation and apoptotic-like cell death in the parenchyma. Further investigations into the molecular mediators of hemorrhagic transformation may reveal new therapeutic targets for this clinically complex problem.

Index Entries: Stroke; neuroprotection; neuromuscular unit; endothelial; blood–brain barrier.

Introduction

Intracerebral hemorrhagic transformation is a complex phenomenon that frequently accompanies ischemic stroke, mainly because of disruptions of the blood–brain barrier (BBB) *(1,2)*. This devastating phenomenon can occur spontaneously, or can be triggered after reperfusion. Because hemorrhagic transformation may be related to reperfusion injury, the increasing use of thrombolytic stroke therapy may lead to a growing incidence of this serious problem *(3–6)*. In this article, we will examine the molecular triggers that mediate this multi-

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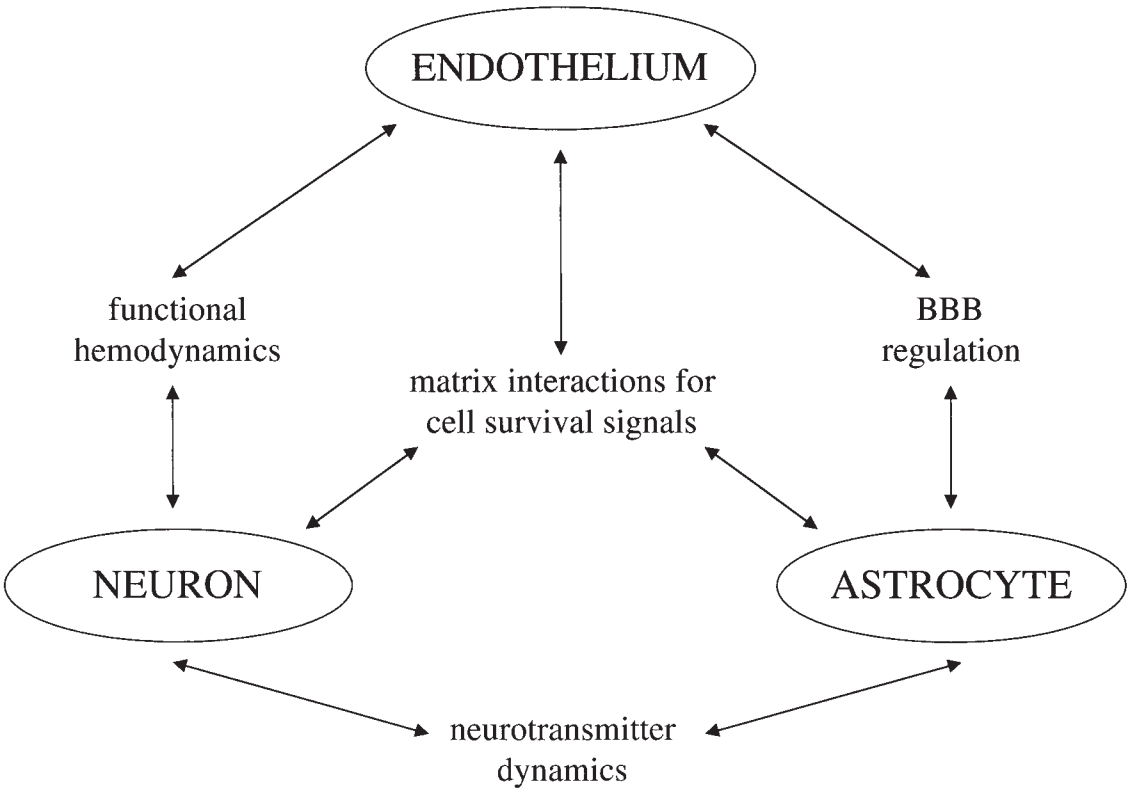

Fig. 1. Schematic of neurovascular unit demonstrating functional interactions between three major cell types: neuron, astrocyte, and endothelium. These interactions underlie a myriad of homeostatic relationships; major ones include neurotransmitter dynamics, hemodynamic coupling, and maintenance of the BBB.

factorial phenomenon, and will relate these mechanisms to perturbed cell-cell and cell-matrix signaling within the concept of the neurovascular unit.

The BBB and Neurovascular Unit

Most studies on brain ischemia originally focused on neuronal tissue or glial cells, which have been long believed to be the primary targets of ischemic insults. However, more recently responses in cerebral microvessels have become an area of increasing interest. An emerging conceptual construct is the neurovascular unit that emphasizes dynamic interactions between the endothelium, adjacent astrocytes and neurons, and the extracellular matrix (ECM) that ties it all together *(7,8).* In this neurovascular unit, the fundamental "building blocks" consist of the endothelial cell of the capillary, the intervening basal lamina/matrix, the encircling astrocytic endfeet that provide a circumferential boundary, and the adjacent neuron. Pericytes and microglia are also likely to contribute. Cell-cell interactions between these components are the functional basis of the neurovascular unit (Fig. 1). Events that occur at the blood-vascular-parenchymal interfaces thus provide the central triggers for initiation of tissue injury. The clinical correlate is disruption of the BBB, with concomitant vasogenic edema and hemorrhage—for example, hemorrhagic transformation *(9–11).*

Within the context of the neurovascular unit, anatomical and functional barriers support vascular integrity and mediate mechanisms of solute transport and transmigration of circulating blood elements. The primary barrier is the BBB itself, which is mainly expressed at the level of capillary and post-capillary venule endothelium. The BBB is essential for the maintenance and regulation of the neural microenvironment. The main characteristic features of BBB endothelial cells are an extremely low rate of transcytotic vesicles and a restrictive paracellular diffusion barrier. The BBB is composed of tight inter-endothelial-cell junctions for fluid retention within the plasma space *(10,12).* Structurally, tight junctions form a continuous network of parallel intramembrane strands of protein connected to the internal actin cytoskeleton *(13).* Although they provide a diffusion barrier, tight junctions are also capable of rapid modulation and regulation *(14).* There are three integral transmembrane proteins (claudin, occludin, and junction adhesion molecule)as well as cytoplasmic accessory proteins that belong to the zona occludens family and others such as AF6 and cingulin. Tight-junction functionality is also related to the ensheathing astrocytic end-feet, which play an essential role in maintaining the BBB phenotype *(13).* In addition to the BBB, a second permeability barrier is basal lamina, a specialized part of the extracellular matrix ECM that connects the endothelial-cell compartment to the adjoining cell layers and the smooth muscle of the media *(15).* Constituent ECM components include type IV collagen, laminin, fibronectin, entactin, thrombospondin, various proteoglycans, and heparan sulphates *(15–18).* Cell signals via the matrix help regulate the BBB and serve to protect the neuronal microenvironment *(19).* Hemorrhagic transformation is linked to processes that alter the integrity of the BBB and basal lamina matrix. Loss of barrier integrity then promotes the infiltration of inflammatory cells and fluids that mediate edema and amplify cell death *(20).*

Clinically, multifactorial mechanisms including hypertension, vascular aging, diabetes, hyperlipidemia, anticoagulant use, and excessive alcohol intake may mediate cerebral hemorrhage by targeting the blood vessels and compromising microvascular integrity *(21).* Most experimental focal stroke models do not mimic many human stroke risk factors (e.g., hypertension, diabetes, or homocysteine) and age-dependent vascular disturbances, which may in part explain the relatively rare occurrence of large hemorrhage in animal models compared with humans in the historical literature *(21).* However, emerging data suggest that under optimal experimental conditions, hemorrhagic transformation can be modeled in animals with severe brain-tissue damage, late reperfusion, and the utilization of clot-based as opposed to mechanical methods of arterial occlusion *(2,5).* Fundamentally, the loss of microvascular integrity may connect the experimental findings of blood–cell extravasation to clinical hemorrhagic transformation. The accumulating experimental evidence now indicates that many mediators involve hemorrhagic transformation after ischemic stroke. In this article, we focus on four potential molecular triggers including oxidative stress, inflammation and leukocyte infiltration, vascular response, and extracellular proteolysis within the context of the neurovascular unit. As further insight is gained into these complex reactions to injury within the endothelium, astrocyte, and neuron, new strategies may emerge to circumvent the high risk of hemorrhage in thrombolytic stroke therapy.

Parenchymal Injury After Hemorrhage

Once hemorrhage has occurred, secondary processes of parenchymal damage and cell death will follow ensue. Although a complete review of the complex pathways of hemorrhagic brain injury are beyond the scope of this paper and the reader is referred to other reviews for in-depth details *(22–24),* a brief survey of the main categories of pathology seems warranted. Overall, secondary injury can be classified into three main overlapping pathways: mechanical, ischemic, and those

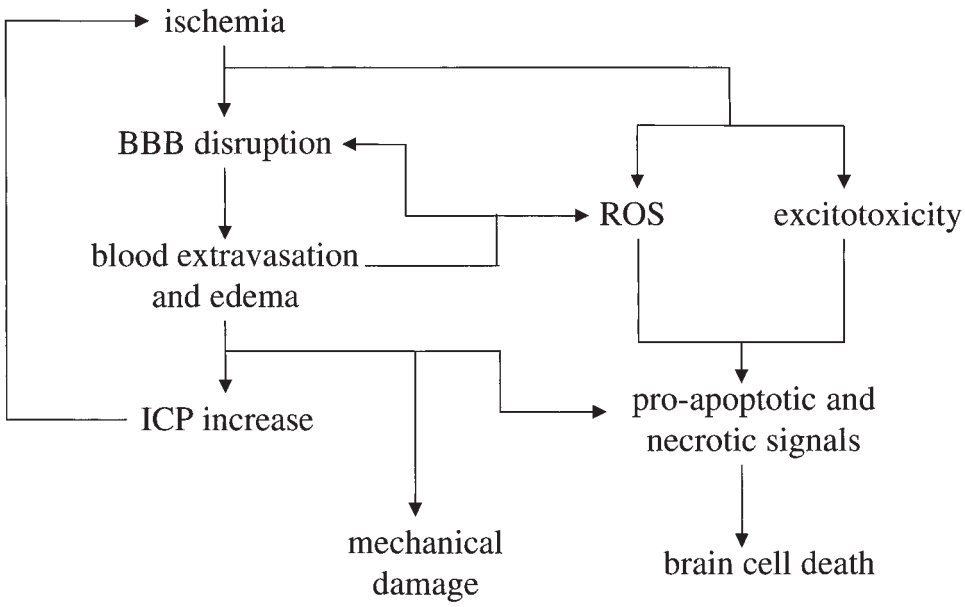

Fig. 2. Simplified schematic representing the multiple cascades of tissue injury that are initiated after hemorrhage. Major pathologic categories include mechanical damage, secondary ischemia, and cytokine and/or toxic-factor signaling that triggers downstream cell-death pathways.

mediated by extravasated cytokines and activated blood elements (Fig. 2).

The first result of hemorrhage within the closed confines of the cranium would be mechanical damage of extravasating blood caused by the shear stress, hemodynamic pressure, and persistent compression onto the adjacent brain. In this regard, there may be considerable overlap in terms of cell-injury mechanisms with what occurs in brain trauma *(25)*. Broadly speaking, excitotoxic, oxidative stress, and apoptotic-like cell-death processes should be recruited, and experimental models of mechanical dilation within the brain has documented these events *(22)*. Emerging results from brain-trauma studies should yield parallel strategies for treating this mechanical aspect of cerebral hemorrhage. For example, recent studies in mouse models of trauma showed that apoptotic death-receptor ligands trigger caspase-dependent and caspase-independent neuronal-cell death *(26,27)*.

A second, and perhaps more controversial, question is whether secondary ischemia occurs around the developing hemorrhage. Rodent models of brain hemorrhage show peripheral ischemia *(28)*. However, clinical neuroimaging studies show equivocal data, with evidence for *(29)* and against *(30)* adjacent ischemia secondary to cerebral hemorrhage. At least in theory, there may be several plausible pathways by which ischemia may occur. Mechanical pressure from hemorrhage can raise intracranial pressure. Additionally, perihematomal edema is a well-established finding that can potentially induce microvascular compression and concomitant reductions in cerebral perfusion. Because there are many vasoactive components in blood as well as lysed blood cells, secondary vasoconstriction may amplify this ischemic reaction within brain tissue. Similar pathways are a major focus of ongoing research in the closely related problem of vasospasm after subarachnoid hemorrhage *(31)*.

Finally, there might be multiple "toxic" factors present in activated blood components that flow into brain after hemorrhagic transformation. For example, basal glutamate levels in

blood are high (50 μ*M*) compared to cerebral extracellular levels (5 μ*M*) *(32).* In a brain that is already damaged by pre-existing ischemia, glutamate homeostasis may be disrupted—e.g., glutamate reuptake transporters that may be inhibited or even reversed *(33).* Thus, extra glutamate that pours in from the blood may amplify excitotoxic brain injury. Similarly, other pro-apoptotic mediators may also play important roles. The common blood component thrombin can modulate neuronal apoptosis, whereas low doses of thrombin-protected neurons from oxidative stress in vitro, higher levels were pro-apoptotic in vitro and in vivo *(34,35).* Plasma levels of prothrombin are high (1-5 μ*M*) so that, upon hemorrhage, a large source of potentially pro-apoptotic factors can be released into brain locally. In addition to thrombin, elevated levels of the pro-death cytokine tumor necrosis factor (TNF)-α and the death ligand soluble FasL may also trigger cell death after hemorrhage *(36,37).* Finally, extravasated blood will release iron and hemoglobin as cells decay *(41).* Hemoglobin is neurotoxic *(38,39)* via pathways of caspase activation and direct oxidative stress *(28).* Indeed, the addition of $FeSO_4$ induces apoptosis in neuronal-cell cultures *(40),* and local injections of $FeCl_2$ into brain in vivo leads to extensive cerebral injury *(41).* Thus, locally elevated blood-decay products after intracerebral hemorrhage may mediate secondary brain injury. Taken together, the evidence of hemorrhagic transformation mediated secondary injury is strong and provides a compelling rationale for investigating the molecular triggers that initiate this process.

Triggers of Hemorrhage After Cerebral Ischemia

Oxidative Stress

Although the precise mechanisms that mediate BBB disruption and hemorrhagic transformation are complex, there is likely to be a strong correlation with deleterious pathways of reperfusion injury. Oxidative stress occurs very early after the onset of ischemia/reperfusion injury via overproduction of reactive oxygen species (ROS), and oxidative damage to lipid-rich membranes in the BBB usually leads to vascular leakage and rupture in ischemic brain tissue *(42).* Arachidonic acid released from brain phospholipids during ischemia/reperfusion is a major source of free radicals and a putative mediator of the BBB disruption and brain edema *(43,44).* Regardless of the source and final pathways involved, membrane damage to the vasculature remains a central event, as evidenced by the ability of membrane "resealing" immunoliposomes to decrease hemorrhagic transformation in a rat model of focal stroke *(45).*

In brain endothelial-cell cultures, hypoxia/reoxygenation significantly increases paracellular permeability between adjacent cells *(14).* Immunofluorescence measurements have showed that increased paracellular permeability is correlated with a redistribution of tight-junction proteins from plasma membrane to cytosolic locations in a process that is dependent on the loss of energy-dependent homeostasis *(14).* More specifically, perturbations in the continuity of ZO-1 proteins have been implicated *(46).* In the context of the neurovascular unit, the importance of cell-cell interactions are underscored by the observation that tight-junction leakage and loss of transcellular electrical resistance in these in vitro models were ameliorated when endothelial cells were co-cultured with astrocytes *(47,48).* The precise mechanism by which ROS perturb tight-junction protein function warrants further study.

In animal stroke models, similar findings demonstrate that ROS are capable of directly injuring the endothelium *(49,50),* and that antioxidants provide a protective effect *(51).* After ischemia, free radical scavengers significantly reduced infarction size and BBB leakage *(52,53).* Mice that lack copper/zinc-superoxide dismutase (SOD1) are highly susceptible to focal cerebral ischemia-reperfusion, with exacerbated vasogenic edema and higher mortality than wild-type animals *(54).* More recently,

clot-based embolic stroke models have been developed, and free radical spin traps effectively reduce spontaneous hemorrhage as well as thrombolysis-induced hemorrhagic transformation *(55,56).* In most of these studies, reduced hemorrhage is obtained in parallel with increased brain-tissue salvage and improved neurological outcomes *(56).* It is possible that reduced hemorrhage after antioxidant treatment may be secondarily related to a reduction of brain-tissue infarction. Nevertheless, taken together, these data strongly demonstrate the involvement of oxidative stress in cerebral hemorrhagic transformation, and suggest that clinical approaches for combining antioxidants and free radical scavengers with thrombolytic agents may be useful.

Neuroinflammation and Leukocyte Recruitment

In addition to direct attack on membrane and protein components of the BBB, oxidative stress may also lead to other cascades of parenchymal pathophysiology with secondary effects on BBB function *(9,10).* For example, ROS is a major stimulator of inflammatory cytokine production and protease secretion by microglia, leukocytes, and resident cells of the neurovascular unit *(57,58).* As these neuroinflammatory mechanisms become activated, alterations in cytokine profiles, adhesion-molecule expression, and tight-junction components mediate further vascular leakage.

Many reports have implicated cytokines in BBB leakage. Direct cerebral injection of two known inflammatory mediators (TNFα and IL-1) leads to massive BBB leakage and edema in experimental models *(59,60).* TNFα and IL-1 can be secreted from activated astrocytes, which then induce the release of nitric oxide (NO) from adjacent endothelium *(61).* Nitric oxide (NO) mediates increased permeability in the BBB, as demonstrated by an in vitro study of porcine-derived cerebral endothelial cells, in which hypoxia-induced BBB leakage is reversed by inhibitors of NO synthase (NOS) *(62).* In addition to diffusible mediators, cytokines can also influence the expression of membrane-adhesion molecules that mediate leukocyte recruitment.

Because postischemic neuroinflammation is a progressive and interactive process, which largely depends on the activation, expression, and secretion of pro-inflammatory mediators from both cerebral and peripheral cells, leukocyte-microvessel interactions—and thus, massive infiltration of leukocytes into the brain—play dominant roles *(63,64).* Interactions between neutrophils and activated endothelium may also mediate inflammation. Under basal physiological conditions, the brain microvascular endothelium acts as a barrier to the immune system that limits the entry of polymorphonuclear (PMN) neutrophils, lymphocytes, and other leukocytes. PMN leukocytes are often the first hemopoietic cells to infiltrate into the brain following ischemia, and contribute to the development of secondary damage by causing capillary plugging, microvascular permeabilization, edema, and hemorrhage via secreted free radicals, cytokines/chemokines, lipid-derived mediators, and proteases *(65–67).*

Leukocyte-microvessel interactions are critical to the neuroinflammatory response, and much has been learned about the complex processes that involve a cascade of several steps including initial contact, rolling, firm adhesion, and ultimately transmigration into the cerebral compartment. The initial capture is mediated by endothelial adhesion molecules, including endothelial (E) selectin, platelet (P) selectin, and vascular-cell adhesion molecules (VCAM-1). The selectin family is comprised of three glycoproteins, and critically mediates leukocyte-endothelial-cell and leukocyte-platelet adhesive interactions. E- and P-selectins demonstrate an inducible response—they are upregulated by activated endothelial cells within the first few hours of cerebral ischemia *(68),* whereas leukocyte (L) selectin is constitutively expressed on leukocytes. E- and P-selectins facilitate the rolling and transient tethering of leukocytes to endothelial cells *(10,64).* E-selectin expression can be induced by cytokines such as TNFα and interleukin-1β *(69),* whereas P-selectin is rapidly

translocated to the cell surface after stimulation by thrombin, histamine, activated complement, and superoxides *(70,71),* all of which would be highly relevant in ischemic brain. In addition, various leukocyte chemoattractants released at the site of injury are believed to provide the driving force for leukocyte movement across the BBB *(72–74).* A more detailed examination of chemokines in brain inflammation has been previously reviewed *(75).*

Increased leukocyte migration alters the molecular organization of the tight-junction complex, reorganization of the actin cytoskeleton, and BBB leakage *(76,77).* In an in vivo study, Bolton et al. demonstrated that leukocyte recruitment following IL-1β injection into the brain can trigger signal-transduction cascades, leading to junctional disorganization with a breakdown of key components such as occludin and ZO1 *(46).* A specific endothelial-cell response occurred in subsets of vessels that underwent extensive leukocyte adhesion and cuffing. This response was characterized by increased staining for phosphotyrosine, loss of the tight-junction proteins occludin and ZO1, and a redistribution of vinculin, an adherent junction proteins. Positive feedback may also occur via TNFα and/or IL-1-induced upregulation in the intercellular adhesion molecule ICAM-1, which amplifies leukocyte transmigration even more *(78).* ICAMs are barely detectable in the normal brain, but become rapidly upregulated after cerebral ischemia. The intracellular signaling pathways resulting from ICAM crosslinking are beginning to be understood. These pathways involve activation of tyrosine kinases, Rho, phosphorylation, and reorganization of the endothelial actin structures, and downstream induction of calcium-dependent signaling via protein kinase C *(79,80).* Indeed, it has been proposed that calcium modulation may provide a potential therapeutic target against ischemic edema and hemorrhage *(81).* Finally, protease activity resulting from leukocytes binding to activated endothelium may also lead to proteolytic degradation of catenin, a component of the endothelial cell-to-cell junction *(82).* Taken together, these multifactorial events following leukocyte-cerebral endothelial-cell adhesion mediate opening of the BBB and cause further damage to microvascular endothelial cells as well as edema and hemorrhagic transformation.

Vascular Responses

Fundamentally, the cerebral microvessel is the proximal target of the ischemic insult, and the primary locus for vascular leakage. As detailed previously, endothelial activation with attendant responses in cytokines, adhesion molecules, and inflammatory infiltration play coordinated roles in triggering BBB disruption and hemorrhagic transformation. In addition to these mediators, highly conserved vascular reactions to injury also play important roles. In particular, the vascular endothelial growth factor (VEGF, also known as vascular permeability factor) has been implicated in endothelial-cell proliferation, permeability and angiogenesis in the reaction to brain injury *(83,84).* Focal ischemia in the rodent induces VEGF expression at 1–3 h, with a sustained peak lasting up to 24–48 h after the onset of ischemia *(85,86).* Acute upregulation of VEGF is localized primarily to the ischemic core, where BBB leakage occurs. Intravenous administration of VEGF to ischemic rats 1 h after the onset of ischemia significantly increased BBB leakage, hemorrhagic transformation, and ischemic lesions *(83).* Treatment with a soluble VEGF receptor chimeric protein, Flt-(1–3)-IgG VEGF, which inactivates endogenous VEGF, significantly decreased brain edema and infarction in a focal ischemia model in mice *(87).* Therefore, acute inhibition of endogenous VEGF may have therapeutic potential in early BBB leakage and hemorrhagic transformation after ischemic stroke. However, targeting the VEGF system requires a careful balance of the trade-offs between detrimental BBB leakage in the acute phase vs the potentially beneficial aspects of VEGF actions that may mediate angiogenic recovery in the delayed periods after stroke *(84).*

The integrity of mature cerebral microvessels also appears to depend on adhesion recep-

tors that link cellular components to specific ligands within the basal lamina/ECM. Integrin adhesion receptors connect endothelial cells to components of the underlying basal lamina. Laminins, a major component of the basal lamina/ECM, are ligands for the integrin heterodimers $\alpha_1\beta_1$ (VLA-1) and $\alpha_6\beta_4$. After experimental focal cerebral ischemia, microvascular expression of integrins $\alpha_1\beta_1$ and $\alpha_6\beta_4$ become rapidly reduced relative to their ligands reflecting an early loss of connective integrity between the vessel wall and the surrounding matrix with adjacent astrocytes *(88,89).* This early reduction in $\alpha_6\beta_4$ expression may be consistent with the astrocyte endfeet swelling that is a cardinal feature of early ischemia in brain *(92).* A more gradual loss of laminin-1, laminin-5, cellular fibronectin, and collagen IV antigens within the basal lamina/extracellular matrix (ECM) also occurs, and this may reflect complicated processes of secondary injury that may be targeted *(88,91,92).* Several experimental studies have demonstrated a significant correlation between the development of hemorrhagic transformation and regional loss of basal lamina markers within the acute phase after focal strokes *(91,93,94).* Further investigations into the mechanisms that lead to these vascular reactions may reveal new therapeutic targets for the treatment of edema and hemorrhage.

Neurovascular Matrix Proteolysis

The classical concepts of reperfusion injury involve ROS and oxidative stress that degrade protein and lipid components vital to BBB function, as discussed previously. More recently, specific proteolytic actions in the vascular matrix have also been shown to participate in the process of hemorrhagic transformation after reperfusion injury. A seminal study from Gasche, Chan, and colleagues showed that loci of ROS production were highly correlated with focal areas of gelatinase protease activity in vulnerable microvessels within the ischemic mouse brain *(95).* Because collagen IV is a target of gelatinase enzymes, degradation of vascular matrix substrates may be the mechanistic link that weakens basal lamina integrity during reperfusion injury.

The mechanisms responsible for basal lamina degradation and increased vascular permeability are not well-defined, but may involve extracellular proteolysis. The key representatives of extracellular proteolysis in the brain are the serine proteases, plasminogen activator (PA), matrix metalloproteinases (MMPs), and proteases secreted by activated PMN leukocytes *(9,10,96,97).*

Recently, there has been an emphasis on the possible role of the zinc-dependent MMPs, which are upregulated after cerebral ischemia and reperfusion, and are associated with brain-tissue damage and hemorrhagic transformation. In mouse, rat, and baboon models of cerebral ischemia, the expression of several MMPs is significantly increased after ischemic onset *(98–103).* Studies suggest a deleterious role for MMPs. MMP injection into the brain results in cell death and inflammation *(104).* Treatment with MMP inhibitors or MMP-neutralizing antibodies reduces edema and infarction in rat and mouse models of stroke *(99,105–107).* Recently, it was demonstrated that MMP-9-knockout mice had significantly smaller lesion volumes compared to wild-type mice after permanent and transient focal ischemia, emphasizing the central role of this protease, at least in mouse systems *(98,99).*

In the context of hemorrhagic transformation after cerebral ischemia, MMPs may degrade vascular basal lamina, weaken vessels, and predispose them to leakage and rupture. In experimental studies, the activation of MMP-9 and degradation of critical protein components of cerebral blood vessels have been correlated with the development of hemorrhage and edema *(93,101).* In a recent study, pharmacological inhibition of MMPs significantly decreased the incidence of hemorrhage in a rabbit model of embolic stroke *(98).* It has been suggested that MMP activation and BBB disruption are intimately associated with the generation of reactive radicals *(95).* Interactions between oxidative stress and the proteolytic cascade may ultimately mediate the progression of

edema and infarction. A targeted knockout of the MMP-9 gene ameliorated matrix degradation and subsequent BBB leakage *(98).* Elevated cytokines after stroke may also be the triggers in upregulating MMPs *(109).* In a hypoxia/reoxygenation (H/R)model in cultured human brain microvascular endothelial cells, we recently found that the MMP inhibitor BB-94 significantly reduced the increase of MMP-2 and MMP-9 levels in media, and decreased the degradation of coating matrix fibronectin induced by H/R. Importantly, BB-94 also inhibited H/R induced cell death, indicating that elevated MMP degrades the extracerebral matrix and may thus mediate cell death as well *(110).* In addition, since the promoter regions of most MMPs include AP-1 and NF-κB sites *(111),* MMP elevations in post-injury are likely to be mediated by MAP kinase pathways *(112)* or oxidative stress *(113).*

The plasminogen-plasmin system may also play a role following cerebral ischemia in the fibrinolysis of vessel-occluding clots and in the proteolysis of FCM components, which potentially contribute to brain edema and bleeding complications. After cerebral ischemia, increased plasminogen activation was observed in the ischemic hemisphere. This activation may promote early secondary edema formation as well as secondary hemorrhage after ischemic stroke *(114–116).* One potential mechanism involved is that the elevated tissue plasminogen activator (tPA) or uPA can activate MMPs, then might further lead to BBB disruption and hemorrhagic transformation.

There are multiple interactions between the PA and MMP axis *(9,96).* Plasmin can activate MMP-1 and MMP-3, converts the zymogen of MMP-9 to the active form, and plasmin inhibitors as well as antibodies to uPA inhibit the conversion of pro-MMP-2. In the context of cerebral ischemia, these interactions become critically important because tPA is used to lyse clots after stroke. MMPs may contribute to damaging processes of extracellular proteolysis after ischemia. If the addition of exogenous tPA can further upregulate the MMP response, these events may be the cause of some of the negative complications associated with tPA stroke therapy *(9,96).*

The functional outcome of patients with acute ischemic stroke is improved if tPA is infused up to 3 h after the onset of stroke symptoms. However, one of the major risks of tPA is the increased rate of symptomatic hemorrhage if administered after the 3-h period *(117–119).* A recent experimental study showed that MMPs were implicated because treatment with a broad spectrum MMP inhibitor BB-94 significantly decreased the rates of hemorrhage induced by tPA in a model of embolic focal ischemia *(108).* In our recent study, tPA-induced hemorrhage volumes were also significantly reduced by co-treatment with BB-94. Importantly, it was shown that tPA treatment significantly elevated the levels of MMP-9 after ischemia *(120).* These data suggest that hemorrhagic complications of tPA therapy may be associated with the linkage between the PA and MMP systems. Recent clinical data also indicate a relationship between MMP-9 levels and subsequent hemorrhage in stroke *(121).* The interaction between tPA and MMP-9 may provide a useful framework for delineating new therapeutic targets. Co-administration with thrombolytics in the treatment of stroke has becoming a considerable of importance. Some pharmacologic agents have shown promise in animal models of stroke by reducing ICH or extending the therapeutic time frame. These agents include MMP inhibitor *(108,120),* free radical scavengers *(56,95),* glycoprotein (GP) IIb/IIIa platelet-receptor antagonist SM-20302 *(2),* and immunosuppressant tacrolimus *(122).*

Interactions and Conclusions

In clinical stroke, the sometimes overwhelming complication of hemorrhagic transformation in the face of potentially beneficial thrombolytic therapy is a major challenge. The historical view of reperfusion injury at the organ level has now been replaced by an emerging understanding of the molecular mediators and substrates that are the basis of this phenomenon. In this short

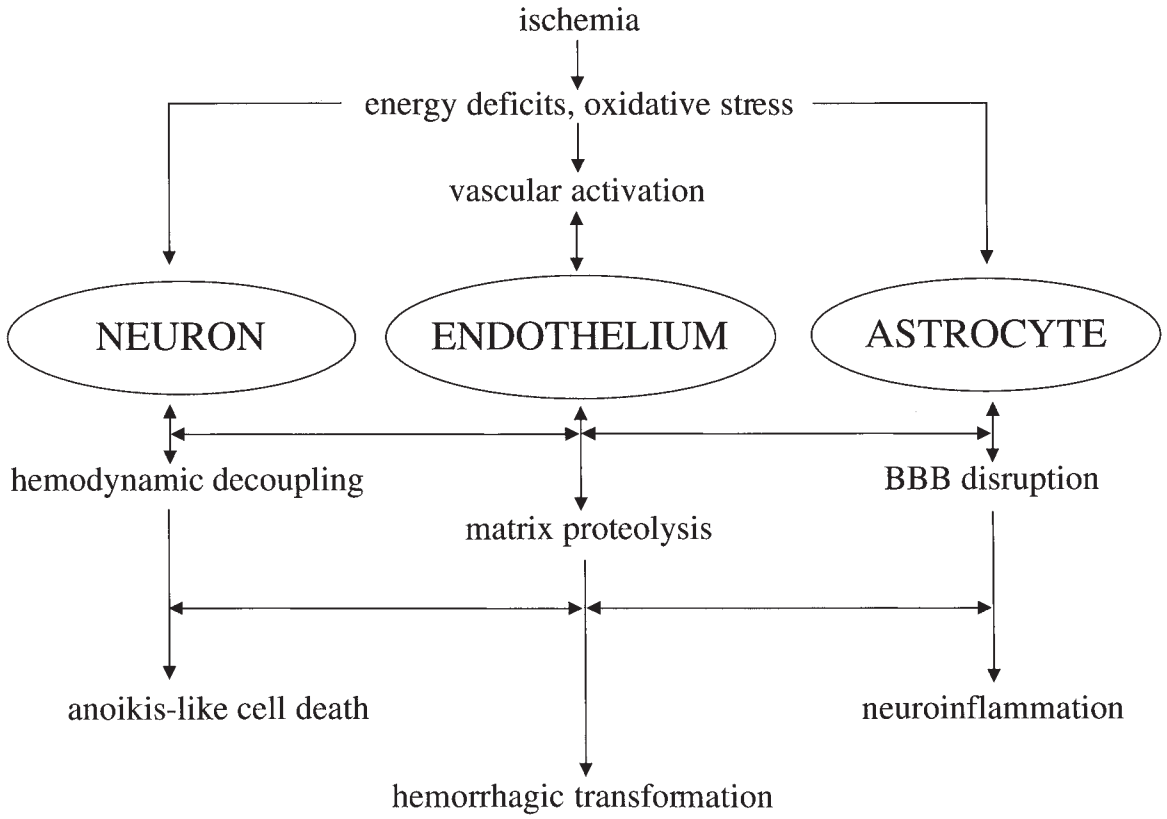

Fig. 3. The neurovascular unit provides a conceptual framework to link multiple triggers and mediators of hemorrhagic transformation in stroke, with subsequent amplification of parenchymal injury and cell death.Once functional relationships within the neurovascular unit are perturbed, hemorrhage can be triggered by overlapping mediators, including oxidative stress, vascular reactions, matrix proteolysis, and neuroinflammation.

review, we have attempted to survey and outline the major triggers of hemorrhagic transformation in brain. Ultimately, the various molecular cascades discussed here demonstrate complex and redundant interactions. For example, alpha-1/beta-1 integrins are involved in MMP regulation *(123)*, and ligation of selectins or cell adhesion molecules directly amplify proteolytic responses in endothelium *(124,125)*. Upstream triggers of oxidative stress will also modify many of the vascular and proteolytic responses outlined above. Thus, targeting a single mechanism will not be sufficient, and combinatorial approaches will be necessary.

Despite the complex overlapping pathways involved, the common theme remains the dynamic signaling that occurs between blood, vascular, and parenchymal compartments when ischemia yields to hemorrhage and further neuronal cell death (Fig. 3). As such, the neurovascular unit provides a conceptual model that can potentially unify the multiple interactions between these elements. A careful delineation of these molecular triggers and mediators should provide new therapeutic targets for this extremely important problem.

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