Cellular mechanisms of neurovascular damage and repair after stroke

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Abstract
The biological processes underlying stroke are complex, and patients have a narrow repertoire of therapeutic opportunities. After the NIH convened the Stroke Progress Review Group in 2001, a paradigm shift took place that shifts the emphasis of stroke research from a purely neurocentric focus to a more integrated view wherein dynamic interactions between all cell types contribute to function and dysfunction in the brain. This so-called “neurovascular unit” provides a conceptual framework that emphasizes cell-cell interactions between neuronal, glial and vascular elements. Under normal conditions, signaling within the neurovascular unit helps maintain homeostasis. After stroke, cell-cell signaling is disturbed leading to pathophysiology. More recently, emerging data now suggest that these cell-cell signaling mechanisms may also mediate parallel processes of neurovascular remodeling during stroke recovery. Since plasticity is a signature feature of the young and developing brain, these concepts may have special relevance to how the pediatric brain responds after stroke.

Keywords
stroke; neurovascular unit; brain remodeling; neurovascular signaling; biphasic response

Introduction
Stroke is now the second leading cause of death and is one of the worst devastating diseases worldwide. While the disease burden is enormous, there are still few therapeutic opportunities for stroke patients. For ischemic stroke, acute treatments are limited to reperfusion with tPA or mechanical catheter-devices. For acute intracerebral hemorrhage, current surgical and medical therapies are extremely limited in efficacy.

For drug discovery in stroke field, early investigations were mostly focused on the prevention of neuronal death. However, the complex pathophysiology of stroke ultimately involves interactions between multiple cell types, so a focus on saving neurons alone might not be sufficient. In 2001, the National Institutes of Neurological Disorders and Stroke convened the Stroke Progress Review Group to explore new directions for stroke research, and at this workshop, the concept of “neurovascular unit” was introduced (http://www.ninds.nih.gov/find_people/groups/stroke_prg/04_2002_stroke_prg_report.htm).

Fundamentally, this concept emphasized that brain function and dysfunction arises from integrated interactions between a network of neurons, glia, and cerebral endothelium. Coordinated responses at the neurovascular interface will mediate not only acute but also
chronic events in ischemic and hemorrhagic brain tissue. During the early acute phase after stroke onset, loss of cell-cell signaling in the neurovascular unit would lead to pathophysiology. During the delayed phase, cell-cell signaling between neurons, glia and vessels may provide the critical neurovascular substrates for functional plasticity and remodeling.

In this mini-review, we will briefly survey this basic idea, i.e. cell-cell crosstalk in the neurovascular unit mediates both acute injury as well as delayed recovery. Although data in this field are still dominated by findings in adult brains, these concepts will surely be even more important in the pediatric brain because plasticity is a signature feature of the young and developing CNS system.

**Neurovascular damage in the acute phase**

Stroke is a heterogeneous spectrum of conditions caused by the interruption of blood vessels supplying the brain. In ischemic strokes, severe blood flow deficits in the core rapidly decrease ATP levels and energy stores. Because cell death processes in this area will occur in minutes, it may be difficult to protect the cells by drug treatments. In contrast, cells in the surrounding region (so-called penumbra) suffer milder insults due to residual perfusion from collateral blood vessels. In the penumbra, cells die more slowly by active cell death mechanisms, and therefore therapeutic approaches are theoretically possible (Figure 1). In hemorrhagic strokes, areas surrounding the central hematoma may also suffer from expanding edema and progressive inflammation and cell death, although the molecular mechanisms are less well defined compared to ischemic strokes.

The fundamental mechanisms of brain cell death in the acute stroke phase are complex. But accumulated data over the past two decades have implicated excitotoxicity, oxidative stress and in some circumstances, apoptotic-like pathways. When brain fails to generate sufficient ATP by reduction of blood flow supply, energy failure occurs and ionic gradients are lost. Glutamate reuptake processes are impaired, and accumulated promotes excessive calcium entry and release. Calcium-dependent synthases and proteases contribute to neuronal death by degrading key cytoskeletal and enzymatic proteins. Abnormality of calcium homeostasis also generates nitric oxide and peroxynitrite, which directly strike neighboring cells. Moreover, mitochondrial functions such as oxidative phosphorylation fail and reactive oxygen radicals are released that further compromise cells by attacking proteins, lipids, and nucleic acids. In parallel with these ionic and free radical pathways, deleterious molecules such caspases may also promote cell death by suicidal endogenous mechanisms. However, most of the cell death pathways outlined here are well documented for neurons. Whether similar mechanisms should be targeted for glial and vascular compartments remains to be carefully assessed.

Beside basic cell death mechanisms, one of the most important facets of early neurovascular damage is manifested as perturbations in blood-brain barrier (BBB) function. The BBB homeostasis is remarkably dependent on endothelial-astrocyte-matrix interactions. Perturbation of the neurovascular matrix (type IV collagen, heparan sulphate proteoglycan, laminin, fibronectin, etc.) disrupts the cell-matrix and cell-cell signaling that maintain neurovascular functions. Many proteinases might contribute to extracellular matrix proteolysis, and the extracellular protease systems become dysregulated under diseased conditions. In particular, roles of the matrix metalloproteinase (MMP) family have been focused in the neurovascular damage after stroke. MMP levels are increased in both experimental models of stroke and stroke patients. Those excessive MMP activities might be deleterious. MMPs can degrade the extracellular matrix that comprises the basal lamina, thus damaging the BBB directly. In experimental stroke models, MMP
inhibition reduces infraction and edema\textsuperscript{14, 15}. In addition to BBB disruption, MMP-induced proteolysis of the neurovascular matrix might also promote programmed cell death by detachment of cells from the extracellular matrix (so-called “anoikis”)\textsuperscript{16, 17}. These findings suggest that MMPs (and other extracellular proteases) mediate neurovascular damage during the acute stages of stroke.

Ultimately, these neurovascular perturbations can also be interpreted as dysfunctional crosstalk between components of the neurovascular unit. Lack of proper signaling between endothelium and astrocytes may underlie blood-brain barrier leakage. Lack of proper signaling between neurons and endothelium may interfere with hemodynamic coupling required for functional brain activation. Lack of proper signaling between neurons and glia may impede normal neurotransmitter release-reuptake kinetics as well as communications along axons. Hence, preventing neuronal death alone will surely not suffice. Saving all these functional interactions across multiple cell types will be required for any truly effective therapy.

Despite convincing experimental evidence, none of above cell death pathways or neurovascular mechanisms have been successfully exploited for treating acute stroke patients. Although many translational barriers are involved\textsuperscript{18, 19}, the heterogeneity of patients and tight timelines during acute pathology makes it difficult to block these early targets efficiently. Therefore, a recent emphasis in the field is beginning to assess opportunities for promoting neurovascular recovery after stroke.

**Neurovascular repair in the chronic phase**

Most stroke patients show some degree of recovery over time. For example, functional MRI studies demonstrate that peri-infarct areas are highly plastic\textsuperscript{20, 21}. Representational areas shift as latent networks are unmasked and parallel circuits are recruited adjacent to damaged regions\textsuperscript{22}. However, the underlying cellular mechanisms for these recovery processes remain to be fully understood.

The primary neurovascular responses during stroke recovery phase are thought to involve angiogenesis and neurogenesis. Molecular mechanisms of angiogenesis and neurogenesis have been evolutionarily conserved so that similar mediators and pathways are involved in both phenomena\textsuperscript{23}. It is now accepted that cell-cell signaling between cerebral endothelium and neuronal precursor cells help mediate and sustain pockets of ongoing angiogenesis and neurogenesis in adult brain\textsuperscript{7, 21, 24-26}. These endothelial-brain interactions comprise the neurovascular niche. Crosstalk between the vascular and neuronal compartments in the neurovascular niche is mediated by an exchange of soluble signals. This phenomenon is partly mediated by the ability of cerebral endothelium to secrete a rich repertoire of trophic factors\textsuperscript{27-29}. In the normal brain, the neurovascular niche defines these complex mechanisms of cell-cell signaling between cerebral endothelium and neural precursors in the subventricular and subgranular zones of ongoing neurogenesis. In the context of post stroke recovery, these close relationships between neurogenesis and angiogenesis are maintained. Neuroblasts migrate along perivascular routes\textsuperscript{30}. Promotion of neurogenesis enhances vascular regrowth and, conversely, angiogenic stimulation enhances neurogenesis\textsuperscript{31, 32}. Angiogenesis in peri-infarct regions have been detected in rodent models of cerebral ischemia\textsuperscript{33} as well as in human stroke\textsuperscript{34}. Hence, brain recovery after stroke comprises interdependent neurovascular plasticity and remodeling processes that recruit multiple common mediators and signals\textsuperscript{23}.

Therapies that can boost these endogenous signals and substrates of neurovascular remodeling might be a new direction for stroke treatments\textsuperscript{35}. However, it remains to be fully elucidated how these approaches can be utilized in clinic. It is worth noting that most
molecular targets for stroke therapy have biphasic roles in stroke pathophysiology. As mentioned in the previous section, during the acute phase, MMPs mediate neurovascular damage through interruption of cell-cell trophic coupling. During the chronic phase, on the other hand, the same mediators may contribute to neurovascular recovery. In a mouse stroke model, endothelial and glial cells in peri-infarct areas showed a secondary elevation in MMP-9, and pharmacological inhibition of MMPs during the delayed phase made outcomes worse. Furthermore, secondary MMP-9 signals co-localized with streams of migrating neuroblasts from the subventricular zone, and MMPs inhibition also blocked the movement of these neuroblasts, which headed towards damaged region. In addition, VEGF and HMGB1 are other examples for biphasic mediators in neurovascular responses after stroke. VEGF increases BBB permeability in the acute phase, and accelerates angiogenesis/neurogenesis in the delayed stroke phase. HMGB1 is normally present in the nucleus, and is released extracellularly in response to ischemic stress. During the acute stroke phase, HMGB1 promotes necrosis and influx of damaging inflammatory cells. In contrast, during the delayed phase, HMGB1 mediates beneficial plasticity and recovery in the neurovascular unit. Likewise, many mediators in the neurovascular unit have biphasic roles, i.e. harmful effects in the acute phase and beneficial in the chronic phase. Hence, neurovascular protection may not be achieved without considering the timing when the initial deleterious responses transits into recovering mechanisms.

Cell-cell trophic coupling in white matter

For the most part, the concept of the neurovascular unit is used to guide investigation in gray matter. However, cell-cell trophic interactions are likely to be important in white matter as well. White matter is vulnerable to ischemic stress and white matter damage is a clinically important part of stroke. Therefore, without considering oligo-protection, we may not be able to protect the brain against stroke insult. Compared to the cellular mechanisms of neurovascular damage/repair in gray matter, white matter pathophysiology remains relatively understudied and poorly understood. However, the idea of the neurovascular unit are now applied to the white matter stroke research.

The main components of white matter are the neuronal axon, oligodendrocyte (myelin), astrocyte, and endothelium. As in the neurovascular unit in gray matter, astrocytes and cerebral endothelial cells work together to maintain blood-brain barrier in white matter. In addition, astrocytes are in close apposition to OLGs within white matter, and couple with OLGs through gap junctions to maintain their functions. Furthermore, astrocyte-derived soluble factors were also reported to nourish oligodendrocyte lineage cells. And, of course, myelin-axon interactions are essential for white matter homeostasis. OLGs not only myelinate axons but also maintain their functional integrity and survival through OLG-specific proteins and/or trophic factor release. Similar to gray matter, during the acute phase of stroke, several deleterious factors/cascades are activated. For example, MMPs are upregulated, and direct attack of MMPs on myelin components affect OLG survival and function. Even if outright cell death does not occur, metabolic dysfunctions in OLGs might still affect the normal replenishment of myelin and synthesis of myelin-associated proteins, which eventually impair myelin-axon interactions.

In the chronic phase, some endogenous responses might work for repairing white matter damage. White matter in the adult brain primarily consists of axonal bundles ensheathed with myelin by mature OLGs, and plays an important role in passing signals between different areas of gray matter. During development, oligodendrocyte precursor cells (OPCs) migrate from the ventricular zone to their final destination, and then differentiate to form myelin sheaths. OPCs are now well-known to widely distributed even in adult brain. And importantly, after brain injury, they are guided to the site for contributing to myelin

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repair. It remains to be fully elucidated how oligodendrogenesis occurs during the chronic phase, however, cell-cell trophic interactions might be involved in this phenomena. As in the so-called neurovascular niche, an oligovascular niche might play an important role in supporting trophic interactions between brain endothelium and OPCs. Hence, cell-cell trophic coupling in white matter may provide an essential mechanism for repairing damaged white matter in the chronic stroke phase.

Conclusions and potential implications for the developing brain

It is now a decade since the concept of neurovascular unit was first introduced. This concept has provided a novel framework for both basic and clinical stroke research. A purely neurocentric focus has been shifted into a more integrated view wherein dynamic interactions between all cell types contribute to function and dysfunction in the brain. As we have seen in this mini-review, multiple mediators induce neurovascular dysfunction in the acute stroke phase. In contrast, the same mediators in turn may underlie neurovascular repair processes in the chronic stroke phase. Understanding cellular mechanisms of the transition zones between injury and repair will give us new directions for stroke treatments. More recently, this concept of the neurovascular unit has grown far beyond its original roots in stroke. The idea that cell-cell signaling in all brain cells comprises the basis for both function and dysfunction is now well accepted in many other CNS disorders (Figure 2).

Although most patients suffering from stroke and neurodegenerative diseases typically comprise an elderly population, lessons from ongoing studies suggest that these concepts should also be relevant for the developing CNS. Crosstalk and signaling between multiple cell types is a central event for CNS development and maturation. Hence, the notion that damaged brain is surprisingly plastic will surely present many parallels with the young and developing brain. Simultaneous neural and vascular remodeling has been observed in neonatal brain injury. And in contrast to the older brain, post-stroke profiles in the developing brain will encompass long periods of recovery, so proper remodeling of the neurovascular unit will be extremely important. However, it is also useful to remember that there will be key differences in how the young and older brain respond to stroke and injury, especially in the context of inflammation. This will be an area that warrants further investigations.

Acknowledgments


References


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**Figure 1.**
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Figure 2.
Trends in neurovascular unit research compiled from publications between 2001 and 2010. An online PubMed search based on the terms “neurovascular unit AND (stroke OR cerebral ischemia OR cerebral hemorrhage)” demonstrated a rapid growth of stroke-related neurovascular unit articles over the past 5 years. The PubMed search based on the terms “neurovascular unit AND (alzheimer OR parkinson OR dementia OR multiple sclerosis OR huntington OR amyotrophic lateral sclerosis OR moyamoya)” suggested that this concept is gradually applying to other CNS disorders.