Review

Neuroprotection targeting ischemic penumbra and beyond for the treatment of ischemic stroke

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Neuroprotection to attenuate or block the ischemic cascade and salvage neuronal damage has been extensively explored for the treatment of ischemic stroke. In the last two decades, neuroprotective strategy has been evolving from targeting a signal pathway in neurons to protecting all neurovascular components and improving cell–cell and cell–extracellular matrix interaction that ultimately benefits the brain recovery after ischemic stroke. The progression from potentially reversible to irreversible injury in the ischemic penumbra has provided the opportunity to develop therapies to attenuate the ischemic stroke damage. Thus, the ischemic penumbra has been the main target for the current neuroprotective intervention. However, despite our increasing knowledge of the physiologic, mechanistic, and imaging characterizations of the ischemic penumbra, no effective neuroprotective therapy has been found so far for the treatment of ischemic stroke. The current acute neuroprotective approach focusing on the damaging mechanisms at the ischemic penumbra is greatly limited by the rapid evolution of the deleterious cascades in the ischemic penumbra. Neuroprotective intervention attempts to promote endogenous repairing in the transition zone of the penumbra for the therapeutic purposes may overcome the unrealistic therapeutic windows under the current neuroprotective strategy. In addition, increasing evidence has indicated ischemic stroke could induce long-lasing cellular and hemodynamic changes beyond the ischemic territory. It is unclear whether and how the global responses induced by the ischemic cascade contribute to the progression of cognitive impairment after ischemic stroke. The prolonged pathophysiological cascades induced by ischemic stroke beyond the ischemic penumbra might provide novel therapeutic opportunities for the neuroprotective intervention, which could prevent or slow down the progression of vascular dementia after ischemic stroke.

Keywords: Stroke, Ischemia, Neuroprotection, Penumbra

Stroke is the leading cause of disability and remains one of the most common causes of death in the industrialized countries.[1] Ischemic stroke caused by an embolus or *in situ* thrombus in a brain blood vessel induces pathophysiological cascades that ultimately lead to irreversible brain damage and corresponding neurological deficit. The effort to develop effective therapies for ischemic stroke has achieved several important successes in thrombolytic therapy.[2] The introduction of recombinant tissue plasminogen activator (rtPA) treatment has significantly improved morbidity of ischemic stroke.[3,4] Encouragingly, the patients receiving rtPA were 30% more likely to have minimal or no disability with no increase in mortality despite a 10-fold increase in the risk of intracerebral hemorrhage.[3] Thus, thrombolytic intervention has been the most effective therapy for acute ischemic stroke ever known.[5,6] Unfortunately, more than a decade after the National Institute of Neurological Disorders and Stroke and European Cooperative Acute Stroke Study trials,[3,4] use of rtPA therapy remains limited. Only a fraction of patients who could potentially benefit from thrombolysis are currently treated, with 1.12% in the United States.[7–9] Several factors have been proposed that could contribute to this limitation: the long delay from stroke onset to thrombolysis, irreversible cell damage induced by cerebral ischemia over time; the profile of rtPA treated patients at high risk of hemorrhagic transformation;[10] and the potential detrimental effects of rtPA.[5,11]

Many emerging strategies have been explored in hope of extending the thrombolytic intervention to larger numbers of patients, including the use of novel fibrinolytic agents, combination therapeutics to

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improve efficacy of clot lysis, refined patient selection with advanced neuroimaging technology, and endovascular approaches.[5,12] Neuroprotection for ischemic stroke was defined as any strategy, or combination of strategies, that antagonizes, interrupts, or slows the sequence of injurious biochemical and molecular events that, if left unchecked, would eventuate in irreversible ischemic brain injury.[13] Neuroprotection approach predicts that ischemic brain damage can in part be prevented from evolving into infarction by the intervention that modulates the key aspects of the ischemic cascade (Figure 1).[14] Great effort has been invested to decipher the ischemic cascade and to further the quest for neuroprotective therapy. Of the numerous identified pathways, excessive activation of ionotropic glutamate receptors, intracellular calcium accumulation, over production of free radicals, neuroinflammation, and initiation of apoptotic signaling are believed to play critical roles in ischemic damage. It is predicted that interruption of these cascades could protect at least part of the brain tissue. In addition, neuroprotection has also been extensively studied in other neurodegenerative diseases such as Alzheimer's disease and Parkinson disease.[15,16]

Historically, monotherapy focused on a single pathway and cell type have dominated studies of stroke. The complexity of the ischemic cascade argues against the concept of single target intervention for cerebral ischemia therapy. Blocking a single deleterious event in a single cell type might not be sufficient to change the consequence of a multifactorial ischemic condition. More recently, neuroprotective strategy has been exploring beyond the neurons and investigating the whole neurovascular components of the brain for the treatment of ischemic stroke. The neurovascular unit provides a conceptual model comprised of cerebral endothelial cells, astrocytes, and neurons, along with extracellular matrix that maintains the integrity of brain tissues. This modular concept emphasizes the dynamics of vascular, cellular and matrix signaling in the brain.[17] Strategies looking over neurovascular unit for an integrative answer to ischemic brain damage may provide a more comprehensive and realistic view of ischemic stroke.[18] Thus, neuroprotection has been evolving into a broader concept involving the protection of all neurovascular components and improvement of their interaction that ultimately benefit the brain recovery in term of ischemic stroke and other neurodegenerative diseases.

Neuroprotection Targets Ischemic Penumbra

The concept of the ischemic penumbra has been broadly defined as an area of severely hypoperfused but potentially rescuable around the ischemic core.[19,20] In an ischemic territory, irreversible damage progresses over time from the center of the most severe flow reduction to the periphery with less disturbed perfusion. Upon ischemic attack, a rapid drop in ATP levels causes failure of the Na^+/K^+ ATPase, disruption of the ionic gradients across the membrane, and subsequent cell death within minutes. However, in the surrounding area, defined as the ischemic penumbra, where cerebral blood flow is partially maintained mainly by the collateral blood flow, the tissue is still viable even with the compromised metabolism.

Since first being defined in 1977, the physiologic profiles of ischemic penumbra and the molecular mechanisms that mediate penumbra cell death have been extensively studied.[21] Cerebral blood flow has been mapped and oxygen and glucose consumption rate have been quantified to define the metabolic thresholds and fates of the at-risk areas. Biochemical pathways involving excitotoxicity, free radical production and apoptotic signaling have been dissected. More recently, the neuroimaging tools as positron emission tomography and magnetic resonance imaging have been developed to detect the ischemic penumbra in real-time (see review[19]). The progression from potentially reversible to irreversible injury after focal cerebral ischemia has provided the opportunity to develop therapies to attenuate the ischemic stroke damage. Early thrombolytic intervention can restore perfusion of the ischemic territory, salvage the ischemic penumbral tissue, prevent the growth of infarction, hence reduce the neurological impairment induced by ischemic stroke.[3] On the other hand, neuroprotective approach alone has not been effective in salvaging the ischemic penumbra in clinical practice although it has been shown to freeze the ischemic penumbra without reperfusion in animal model of ischemic stroke.[22] The rapid evolution of the penumbra toward irreversible damage greatly limited the neuroprotective therapy. The future of neuroprotection to salvage the ischemic penumbra as a strategy for acute ischemic stroke remains uncertain.[23]

The ischemic penumbra is highly dynamic in term of the rapid evolution of the deleterious cascades that ultimately lead to infarction when early reperfusion does not occur. In addition to damaging, ischemic cascade also triggers brain repairing process. Biphasic role of the ischemic cascades, such as NMDA receptor and matrix metalloproteinase activation, in brain damaging and repair process at the ischemic penumbra have been found. The ischemic penumbra is not just passively dying over time, but also actively recovering.[19] Increasing evidence has demonstrated that neurogenesis process may persist for surprising long period after ischemic stroke.[20] Therefore, neuroprotective intervention attempts to promote endogenous repairing process for the therapeutic purposes may overcome the unrealistic therapeutic windows under the current neuroprotective strategy. Future dissection

of the molecular cascade in the ischemic penumbral tissue that are responsible for the transition of the ischemic injury and repairing may provide critical insight for the neuroprotective approach to treat ischemic stroke.[19,24]

Ischemic Stroke Triggers Responses beyond the Ischemic Territory

Ischemic stroke induces an increase in intracranial pressure that could compromise global hemodynamics.[25] In addition, given the extensive neuronal network within and between each hemisphere, it might not be surprise that focal cerebral ischemia could trigger prolonged global reaction. Increasing evidence has indicated that occlusion of a cerebral artery could induce both hemodynamic and cellular changes beyond the ischemic territory.

Increasing evidence has shown that neural stem/progenitor cells are induced in the ischemic core at the post-stroke murine and human brain.[26,27] Administration of bone marrow cells promotes the proliferation of neural stem/progenitor cells both in the ischemic core and penumbra area in a mouse model of ischemic stroke.[28] These findings suggest that ischemic core also becomes a potential target as well as ischemic penumbra. In addition, it is well known that the focal cerebral ischemia could induce stem cell proliferation in the subventricular zone and dentate gyrus, areas beyond the ischemic territory. In rodent ischemic stroke models, the proliferation of neural stem cells and other neuronal progenitors in the subventricular zone increase and generate neuroblasts, which migrate to the ischemic territory, morphologically integrate and become functional mature neurons.[29,30] Most important, the neurogenesis after ischemic stroke has been found to survive for several months.[30] Increased proliferation of neuronal progenitor cells has also been shown in the contralateral hippocampus after focal cerebral ischemia in rats,[31,32] with a substantial number of cells differentiating into astrocytes. Consistently, there is also evidence in human for the increase of neuronal progenitor cells and neuroblasts formation in areas distant from the ischemic territory after stroke.[33,34]

Clinically and experimentally, ischemic stroke is followed by both acute and prolonged inflammatory response, involving activation, proliferation, and hypertrophy of microglia and astrocytes. Originally, this reaction was believed to be related to repair at the site of injury. Recently, it has been noted that inflammatory responses play an important role in the development of ischemic injury.[35] Inflammatory responses after focal ischemic stroke have been extensively studied in the primary ischemic area.[36] Reactive astrogliosis is a key component of the cellular response to CNS injury.[35] Considerable evidence indicates that inflammatory mechanisms modulate both proliferation and biosynthetic activities of reactive astrocytes,[37] which exert both harmful and beneficial actions during repair processes in the injured CNS.[35] The activated glial cells produce cytokines, which further stimulate glial cells, gliosis and cytokine production in a self-propagating cycle.[38] Further, ischemic stroke not only causes endogenous parenchymal cell damage, but also involves inflammatory responses that include the infiltration and accumulation of leukocytes, monocytes/macrophages and serum proteins due to breakdown of the blood brain barrier.[39] In addition to the primary ischemic area, inflammatory responses in the remote area to the ischemic lesion have also been indicated. Using middle cerebral artery occlusion (MCAO) model, a delayed neurodegeneration was found in thalamus and substantial nigra, which are supplied by posterior cerebral artery and showed no sign of infarction following MCAO. Neurodegeneration in thalamus and substantia nigra were preceded by TNF-alpha expression, suggesting the role of inflammation in the remote area to the ischemic lesion after stroke.[40] Transient expression of interleukin-6 has been observed in the substantia nigra after MCAO.[41] Further, ischemic stroke induces a global inflammatory action both in the CNS and periphery. Inflammatory biomarkers such as interleukin-6 and matrix metalloproteinases-9 are significantly increased in plasma after stroke.[42]

It is well established that maintenance of energy homeostasis is the fundamental necessity for survival of a single cell and a multi-cell organism. The adenosine monophosphate-activated protein kinase (AMPK) functions as a critical energy sensor that detects and initiates adaptive changes in response to vibrations in energy balance.[43] AMPK activation were found not only in the ischemic territory but also in non-ischemic hemisphere within 24 hours after transient focal cerebral ischemia induced by MCAO.[44] The global activation of AMPK suggests a global metabolic derangements and compensatory responses after ischemic stroke.

As a cerebral vascular event, ischemic stroke induces not only a complex array of pathogenic cellular cascades in the brain parenchyma, but also impairment of autoregulation in the brain vasculature. Transient focal cerebral ischemia has been found to affect the myogenic response of the cerebrovasculature at the acute stage after ischemic stroke.[45–47] Diminished responsiveness to the vasoconstrictor 5-HT, abolition of endothelium-dependent relaxation due to acetylcholine, and diminished distensibility have been found after transient focal cerebral ischemia in experimental stroke models.[45,48] During focal cerebral artery occlusion, the circulatory system can

compensate for the reduction of focal CBF using collateral circulation and autoregulatory mechanisms, which causes hemodynamic perturbations and a redistribution of CBF.[49,50] Flow territory maps in patients with internal carotid artery occlusion showed significant differences in the flow territories of the contralateral internal carotid artery and vertebrobasilar arteries compared with those in the control subjects.[25] In experimental stroke models, transient unilateral hemodynamic stress induces a transient increase in CBF velocities in the opposite hemisphere and delayed cleavage of neuronal caspase 3 in both the ischemic and contralateral side cortices.[51] In addition, endothelial dysfunction has been found in the peripheral mesenteric resistance artery after experimental ischemic stroke in rats.[52] Ischemic stroke could trigger an inflammatory process that has been associated with an increase in cytokine expression in plasma, which might contribute to the impairment of vasculature function.[47] Taken together, both clinical evidence and experimental stroke studies have indicated that ischemic stroke could induce long-lasting pathophysiological responses beyond the ischemic territory (Figure 2).

Ischemic Stroke Triggers Progressive Cognitive Function Decline

Stroke is the most common cause of permanent disability among people in the United States and is associated with a high incidence of deficits in sensorimotor function and cognitive ability.[53] Stroke patients must not only survive the acute stages of infarction, but they must then cope with significant physical, mental, and economic stresses associated with ongoing neurological impairment.

Vascular dementia (VaD) incorporates cognitive dysfunction with vascular disease. Epidemiological studies have shown that the prevalence of dementia in ischemic stroke patients is 4–12 times higher than controls.[54–57] Further, a progressive course of dementia after ischemia stroke has been suggested. Tatemichi *et al.*[58] reported that the incidence of dementia was 6.7% among patients after 1 year of follow-up in patients who were initially nondemented after stroke. Bornstein *et al.*[59] reported that 32% patients who were initially nondemented after stroke developed dementia during 5 years of follow-up. Hénon *et al.*[60] examined a cohort of 169 patients who had been nondemented before stroke onset and reported that the cumulative proportion of patients with dementia was 21.3% after 3 years of follow-up. Altieri *et al.*[61] followed up 191 nondemented stroke patients for 4 years, and found that the incidence of dementia increased gradually with 21.5% patients had developed dementia by the end of the follow-up period. In population-based studies of stroke and dementia, Kokmen *et al.*[62] reviewed the medical records of a sample of 971 patients who were free of dementia before first stroke. The cumulative incidence of dementia was 7% at 1 year, 10% at 3 year, 15% at 5 years, and 23% at 10 years. Desmond *et al.*[63] performed functional assessments annually to 334 ischemic stroke patients and 241 stroke-free control subjects, all of whom were nondemented in baseline examinations, and found a progressive course of dementia with the incidence rate of 8.94 per 100 person-years in the stroke cohort and 1.37 cases per 100 person-years in the control cohort. In two studies based on patients presenting with a lacunar infarction as their first stroke, Samuelsson *et al.*[64] found that 4.9 and 9.9% of 81 patients developed dementia after 1 and 3 years of follow-up, respectively, and Loeb *et al.*[57] found that 23.2% patients developed dementia during an average of 4 years of follow-up.

MCAO in rodents is considered to be a convenient, reproducible, and reliable model of ischemic stroke in humans.[65–67] MCAO typically results in extensive damage in the cortex and caudate putamen, centers for sensorimotor function.[66] Sensorimotor behavior impairment has been extensively studied in ischemic stroke models. Spontaneous partial or complete recovery of sensorimotor function has been reported consistently over time after ischemic stroke.[67–72] On the other hand, different progression of cognitive impairment has been demonstrated in MCAO models. The Morris water maze has been used to study impairment in spatial learning and memory in experimental stroke studies.[68,70,71,73–76] The experimental protocols and indices of performance used in these studies vary considerably; consequently, the conclusions are not always consistent. However, almost no studies have shown recovery of the cognitive impairment over time after MCAO, and a progressive impairment of cognitive function has been suggested.[69,72,73,76] A longitudinal behavior study following transient MCAO in rats demonstrated sensorimotor and spatial memory impairment up to 1 year. Interestingly, no progression of sensorimotor dysfunction was found from the repeated test at 7 month and 1 year after transient focal cerebral ischemia. For the spatial memory test, improvement of performance was found in both stroke and sham animals at 1 year after insult or sham surgery, respectively, as a result of repeated test, compared with the behavior test obtained at 7 months after stroke or sham surgery. However, the improvement in the stroke animals was profoundly less than that of sham animals, suggesting a progression of spatial memory impairment from 7 month to 1 year after ischemic stroke.[69] In similar behavior test paradigm, rats with permanent MCAO showed less improvement of performance in repeated spatial memory test at 1 and 2 weeks after stroke when

$$R_I = \frac{1}{1 + e^{-t}}$$

Thrombolytic Intervention Equation for ischemic Stroke

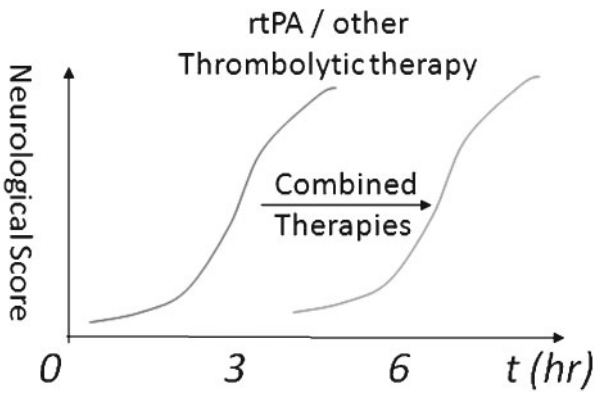

Figure 1 The hypothetical thrombolytic intervention equation for ischemic stroke and the obtained sigmoid curve for thrombolytic therapy derived from experimental stroke studies.[79–81] R_I is the recovery index and t is the time of thrombolytic intervention. The curve demonstrates that the earlier thrombolytic intervention applied, the better neurological score obtained. The initial phase of action for thrombolytic intervention is approximately exponential; then, as reaching the plateau, the beneficial action of thrombolytic intervention disappears. It is predicted that combined therapies such as neuroprotection could shift the sigmoid curve to the right, hence, expends the therapeutic window of thrombolytic therapy.

comparing with shams.[72] The delayed progression of the cognitive impairment argues against the direct contribution of the primary ischemic damage.

Traditional concepts of VaD postulate that cognitive decline in patients with ischemic stroke can result from stroke alone when a large volume of brain is affected by infarcts and overcoming the brain's reserve or compensatory mechanisms. In other words, VaD could be arising from the unmasking effect of the vascular lesion on a previous degenerative process. Epidemiologically, as many of these dementias developed progressively after stroke, the delayed VaD could not be interpreted as the direct deficit induced by the primary cerebral damage. Rather, the progressive course of VaD suggests a progressive degenerative disorder.[62,77]

For more than two decades, neuroprotection to attenuate or block the ischemic cascades has been intensively investigated and numerous agents have been found to be protective in both cell culture and animal models of ischemic stroke. However, the translation of the neuroprotective benefits from the basic research to clinical practice has not been successful.[78] Despite the increasing knowledge of the physiologic, mechanistic, and imaging characterizations of the ischemic penumbra, no effective neuroprotective therapy has been developed for the treatment of ischemic stroke alone or to expand the short therapeutic window of rtPA thrombolytic intervention. Acute neuroprotective intervention targeting the ischemic penumbra is greatly limited by the rapid evolution of the ischemic penumbra. The continuing failure of the translation of neuroprotection to clinical practice has indicated that novel neuroprotective approaches should be adopted. It is clear that ischemic stroke could induce long-lasting pathophysiological cascades beyond the ischemic territory. It is still unclear whether and how the prolonged global response induced by the ischemic cascade contributes to the progression of the vascular cognitive impairment after ischemic stroke. A better understanding of ischemic and restorative brain mechanisms beyond the ischemic penumbra may open new therapeutic options to improve stroke recovery.

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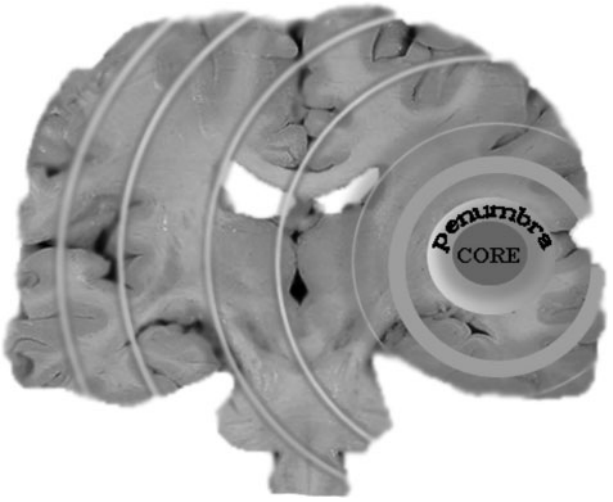

Figure 2 Hypothetical scenario for the progression of ischemic core, penumbra, and global cascade beyond ischemic territory after ischemic stroke. In the ischemic core, a rapid drop in ATP levels causes failure of the Na^+/K^+ ATPase, disruption of the ionic gradients across the membrane, and subsequent cell death within minutes. In the ischemic penumbra where cerebral blood flow is partially maintained, the tissue is still viable even with the compromised metabolism. Acute neuroprotective intervention targeting the ischemic penumbra is greatly limited by the rapid evolution of the ischemic penumbra. A better understanding of ischemic and restorative brain mechanisms beyond the ischemic penumbra may open new therapeutic options to improve stroke recovery.

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