Angiogenesis
A Harmonized Target for Recovery After Stroke

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It is now appreciated that emerging therapeutic strategies for recovery must include the cerebral vasculature and that induction of angiogenesis will stimulate endogenous recovery mechanisms, including neurogenesis, synaptogenesis, and neuronal and synaptic plasticity. These events are all involved in the long-term repair and restoration process of the brain after an ischemic event. Several recent excellent reviews provided detailed information on the mechanisms and molecular targets for angiogenesis after stroke. The purpose of this review is to evaluate the evidence that angiogenesis is a target for recovery after an ischemic stroke.

Angiogenic Response to Ischemic Brain Injury: A Multipurpose Pathway

Early reports of increased angiogenesis in the ischemic border zone of human brain autopsy sections, which was decreased in patients of advanced age, led to interest in the time course and impact of this phenomenon on functional recovery. It is clear that angiogenesis genes are upregulated within minutes of the onset of cerebral ischemia in rodents and angiogenic proteins remain increased in the area of ischemia for days to weeks. It is unclear, however, whether the angiogenic response leads to the development of functional new blood vessels that improve brain function after stroke. Clinical and experimental studies in other vascular beds have emphasized the potential for adverse consequences related to neovascularization. In the diabetic retina, for example, pathological angiogenesis results in hemorrhage, edema, and, ultimately, blindness. In the brain, pathological angiogenesis is implicated in the development of hereditary hemorrhagic telangiectasia.

The correlation between angiogenesis and improved functional outcome after ischemic stroke remains and is seen in both animal models and in human patients with stroke. It is likely that the “proangiogenic state,” induced in response to an ischemic insult, has multiple purposes in the hours to weeks after the injury (Figure). First, the growth factors expressed may promote survival of the endothelial, glial, and neuronal cell types in the penumbral area. Second, the neovascularization may act to remove damaged tissue. In experimental stroke, it was demonstrated that angiogenesis only occurred transiently in the cortex of the ischemic hemisphere, implying that the new vessels were merely part of the “clean up” after stroke rather than a contribution to neurorestoration. Lastly, the proangiogenic state may create a “vascular niche” in which neural stem cells are generated and allowed to migrate.

Growth Factors

Numerous growth factors have been implicated in the recovery process after ischemic stroke and include basic fibroblast growth factor, brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), granulocyte colony-stimulating factor, and other neurotrophins. Neutrophins promote recovery by enhancing angiogenesis, neurogenesis, synaptogenesis, and neuronal plasticity. The most commonly studied proangiogenic molecule in stroke is VEGF. Although touted for its neuroprotective and neurogenic properties, VEGF can worsen edema and hemorrhage after stroke through its enhancement of vascular permeability and this effect is dose-related. However, a great deal of evidence exists that the proangiogenic and vascular permeability effects of VEGF can be “uncoupled.” When this occurs, the protective effects predominate and recovery is promoted. Differential expression of the various isoforms of VEGF may be important in this process after stroke, when VEGF-B promotes cell survival without the predominant vascular permeability and angiogenic effects demonstrated with the better studied VEGF-A isoform.

BDNF is a member of the neurotrophin family that is widely expressed and has many functions extending beyond its neuronal effects. After experimental ischemia, higher levels of BDNF in the brain improved functional recovery, whereas BDNF knock down worsened functional outcome. Mice expressing a mutant variant of BDNF (Met BDNF) had a worse outcome in comparison with wild-type mice after cerebral ischemia and this worsened outcome was attributed to an impaired angiogenic response in animals expressing mutant BDNF. A unique aspect of BDNF biology was the identification of its involvement in the
Crosstalk between the constituents of the neurovascular unit to promote recovery after central nervous system ischemic insults. Additionally, BDNF has been reported to regulate the expression of VEGF. BDNF has been implicated in cardiovascular development and found to induce a robust angiogenic effect in endothelial cells comparable to that of VEGF.

Nonpharmacologic Interventions That Improve Recovery: Effect on Angiogenesis

Exercise/Physical Therapy
It is clear that exercise preconditioning induces tolerance to brain ischemia through many proposed mechanisms, including induction of VEGF and stimulation of angiogenesis and neurogenesis. Physical activity after stroke has also been shown to induce angiogenesis and improve long-term outcome as well. The most important finding in this study, however, was that inhibition of endothelial nitric oxide synthase or angiogenesis (with endostatin) abolished the beneficial effects of exercise on outcome.

Peripheral/Transcranial Magnetic Stimulation
Noninvasive brain stimulation has been used extensively in rehabilitation research and the promise in promoting recovery after brain injury has been reviewed recently. The mechanism of the persistent benefit of this intervention, when combined with physical therapy, is not entirely understood. In an experimental study of middle cerebral artery occlusion in a rat model, electric stimulation resulted in decreased apoptosis and inflammation and increased angiogenesis in the ischemic cortex. These effects were accompanied by a robust increase in neurotrophic factors, including VEGF and BDNF, and the antiapoptotic effect was abolished by inhibiting Akt activation. The importance of angiogenesis was further implicated in the positive response to peripheral stimulation when mice treated with a specific inhibitor of VEGF receptor-2 had decreased neuroblast migration toward the injured area.

Low-Level Laser Therapy
Light therapy has been shown to reduce infarct size and improve outcome when administered at least 6 hours after experimental stroke onset. Experts in the technique have agreed on a critical role of VEGF and angiogenesis to the protection afforded by light therapy. Laser therapy is currently in clinical trials for promotion of recovery after ischemic stroke.

Repurposed Pharmacological Interventions That Improve Stroke Recovery

Statins
Statins have been shown to limit the ischemic insult and improve functional outcome and recovery after experimental ischemia. The observed improved outcome has been attributed to the statins’ ability to induce neurogenesis, neuroprotection, neuroplasticity, and angiogenesis in the ischemic border zone. Data from in vitro and ex vivo angiogenesis research support a biphasic dose-dependent effect of statins in angiogenesis in which lower concentrations have a proangiogenic effect and higher statin concentrations produce an antiangiogenic response in endothelial cells. In a striking contrast to the proangiogenic effect of statins in models of ischemia, statins have been shown to have an antiangiogenic effect in both cancer and inflammation settings. It can be concluded that statins have a dose- and context-dependent modulatory effect on angiogenesis as has been previously reviewed.

Angiotensin II Receptor Type 1 Antagonists
Similar to the statins, reports of the effects of the angiotensin II receptor Type 1 antagonists on angiogenesis have been conflicting. A meta-analysis of clinical trials using these agents in various disease states pointed to a small, but significant, increase in the incidence of lung cancer in treated patients. However, angiotensin II receptor Type 1 antagonists have been shown to be both pro- and antiangiogenic, depending on the situation (ischemia or not) and tissue involved. In a recent set of experiments done in a rat model of ischemic stroke, treatment with candesartan after 3 hours of temporary middle cerebral artery occlusion resulted in improved long-term functional outcomes and a proangiogenic state. This acute treatment was subsequently shown to be associated with increased VEGF expression in both the...
ischemic and nonischemic hemispheres at 24 hours after treatment and increased vascular density in both hemispheres at 7 days. Most belief that angiogenesis only occurs in the ipsilateral hemisphere after stroke and experimental studies compare only the cortical tissue in the 2 sides for evidence to support this claim. It is well known, however, that ischemic injury in the brain can result in diaschisis (distant areas of decreased flow and metabolism) and functional MRI studies in human stroke victims have consistently shown involvement of the contralesional hemisphere in recovery. It is possible that induction of angiogenesis in the striatum of the contralesional hemisphere is important to functional recovery after ischemic stroke by improving recruitment of neuroblasts to the area of recovery.

**Phosphodiesterase Type 5 Inhibitors**

Recent evidence shows that phosphodiesterase Type 5 expression varies among vascular beds and is an important determinant of angiogenic potential, with endothelium expressing the lowest levels of phosphodiesterase Type 5 being the most angiogenic. The phosphodiesterase Type 5 inhibitors, sildenafil and tadalafil, have both been studied in rodent models of ischemic stroke and found to promote functional recovery when initiated at 24 hours after the onset of ischemia. Sensitive imaging techniques revealed that rodents treated with sildenafil after embolic stroke experienced increased angiogenesis and axonal remodeling in the ischemic boundary zone compared with saline-treated controls beginning at 1 week after injury. The authors concluded that the temporal and spatial colocalization of the 2 processes supported the notion that angiogenesis promoted axonal remodeling and, therefore, recovery.

**Growth Factor Treatment**

Many growth factors, including granulocyte colony-stimulating factor and erythropoietin (EPO), have been studied for their neuroregenerative effects but dose-limiting toxicity and drug delivery barriers have tempered their use. EPO has been the most studied and was found to enhance angiogenesis, neurogenesis, and functional recovery after stroke in rats and this was associated with an increase in both VEGF and BDNF expression. A small clinical trial of 328 patients with stroke treated with granulocyte colony-stimulating factor or placebo failed to show a benefit of treatment at 90 days but it may have been underpowered to do so. EPO has been the most studied and was found to enhance angiogenesis, neurogenesis, and functional recovery after stroke and this was associated with an increase in both VEGF and BDNF expression. Clinical trials with EPO, at first promising, were discontinued when increased hemorrhage and mortality occurred in patients with stroke receiving the combination of EPO (within 6 hours of onset) and tissue-type plasminogen activator. Subsequently it was shown that EPO increases matrix metalloproteases, which may have explained this interaction. When administered beyond the first 24 hours after ischemia, however, the data on EPO continue to be encouraging. In a recent study, EPO administered 3 days after focal ischemia increased recovery in affected mice and was associated with increased remodeling of the ischemic boundary zone and contralesional axonal sprouting. The increased expression of proangiogenic genes and proteins in the nonischemic hemisphere is an exciting area for future study.

**Which Came First?**

Spatial and temporal colocalization of angiogenesis and neurogenesis does not prove that angiogenesis is necessary for the neuronal recovery after treatment with the previously mentioned interventions. The best evidence supporting causation comes from a classic set of experiments in the female canary. When testosterone was administered to these songbirds, an abrupt increase in VEGF expression in the vocal control nucleus led to increased angiogenesis and this was followed by a significant increase in BDNF 2 weeks later. The increase in BDNF, primarily from endothelial cells, was shown to significantly increase neuronal migration to the nucleus. When VEGF signaling was blocked with a receptor inhibitor, recruitment of new neurons was impaired. In stroke, although angiogenesis may not be necessary for the neuroproliferative response to ischemia, long-term migration of neuroblasts to the site of recovery has been shown to occur only in areas of newly increased vascularity.

**Consequences of Impaired Angiogenesis**

As commented in a recent review, diabetic and/or hypertensive patients may develop dysfunctional vasculature after stimulation of angiogenesis in the recovering penumbra after stroke, and this can be detrimental. There is strong evidence that hyperglycemia-mediated oxidative damage to microvascular endothelial cells triggers a cascade of events that causes excessive angiogenesis and result in vascular proliferative retinopathy. These immature vessels then break and leak worsening vascular and neuronal damage. The effect of hyperglycemia in the cerebral vasculature, especially after an ischemic event, is less clear. We reported extensive vascular remodeling and arteriogenesis in the pial vessels in Goto-Kakizaki rats, a lean and mild model of Type 2 diabetes. Also, cerebrovascular permeability, VEGF, and BDNF levels are increased in this model. After stroke, these diabetic animals bleed into the brain and perform poorly on neurobehavioral tests. Prevention of pial remodeling by glycemic control or inhibition of matrix metalloproteases reduces vascular damage and improves neurological outcome. Although antiangiogenic therapy has been successful in reducing damage when applied locally to the retina, systemic use is associated with increased blood pressure, a perilous pursuit in patients at risk for stroke.

In hypertension, it is well established that cerebrovascular remodeling occurs but our knowledge of how hypertension affects angiogenesis is limited. Increased angiogenesis occurs in the mesenteric microvascular network of spontaneously hypertensive rats, but it is followed by a phase of increased pruning. The time course and significance of this phenomenon in the cerebral vasculature has yet to be determined.

**Conclusions**

Angiogenesis occurs after stroke and can be augmented by pharmacological and nonpharmacological means. Although new blood vessel growth may be transient in the ischemic boundary zone after injury, the association of angiogenesis with neuronal plasticity, especially in the contralesional hemisphere, could result in enduring recovery. The beneficial effects of angiogenesis may be negatively impacted by...
premorbid disease, as is seen in diabetes and hypertension, and this area requires further investigation. Progress in this area has been limited by reliance on the nonischemic hemisphere as a convenient control, focus on the ischemic boundary zone, and failure to include an assessment of pre-existing vascular remodeling. Harnessing the restorative power of the vasculature in the entire brain, in the face of vascular diseases, should be a high priority for future study.

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