Targets for Vascular Protection After Acute Ischemic Stroke
Susan C. Fagan, PharmD; David C. Hess, MD; Elizabeth J. Hohnadel, MS; David M. Pollock, PhD; Adviye Ergul, MD, PhD

Background—Vascular damage caused by cerebral ischemia leads to edema, hemorrhage formation, and worsened outcomes in ischemic stroke patients. Therapeutic interventions need to be developed to provide vascular protection. The purpose of this review is to identify the pathophysiologic processes involved in vascular damage after ischemia, which may lead to strategies to provide vascular protection in ischemic stroke patients.

Summary of Comment—The pathologic processes caused by vascular injury after an occlusion of a cerebral artery can be separated into acute (hours), subacute (hours to days), and chronic (days to months). Targets for intervention can be identified for all 3 stages. Acutely, superoxide is the predominant mediator, followed by inflammatory mediators and proteases subacutely. In the chronic phase, proapoptotic gene products have been implicated.

Conclusions—Pharmacological agents designed to target specific pathologic and protective processes affecting the vasculature should be used in clinical trials of vascular protection after acute ischemic stroke. (Stroke. 2004;35:000-000.)

Key Words: cerebral ischemia, focal ▪ clinical trials ▪ free radicals ▪ ischemia ▪ pharmacology

During focal cerebral ischemia, cerebral blood vessel damage occurs early and in a progressive fashion.1 If prolonged, cerebral edema and hemorrhagic transformation will ensue. Reperfusion through damaged cerebral blood vessels is likely to further increase the ultimate tissue damage caused by ischemic stroke. There has been a recent call to develop means to protect the vasculature to improve stroke outcome.2 Neuroprotection is a well-studied therapeutic intervention for acute stroke, with many targets identified.3 The purpose of this review is to identify targets on which to focus for development of vascular protection after acute ischemic stroke.

Chronic vascular protection has been approached traditionally through enhancement of endothelial function, prolongation of endothelial cell survival, and suppression of thrombosis and anti-inflammatory effects within the vasculature.4 In practical terms, these mechanisms translate into a reduction in vascular events including myocardial infarction, stroke, and claudication.5 Of course, any strategy proven to reduce the incidence of fatal and nonfatal vascular events could be said to be “vascular protective,” including antithrombotic and antihypertensive therapies, but historically, this moniker has been reserved for agents with direct beneficial effects on vascular endothelium, often beyond their primary pharmacological action.5–10 The most prominent agents in this realm include the statins, angiotensin-converting enzyme inhibitors, and angiotensin receptor blockers. Whether vascular protection strategies will prove useful in the initial hours after stroke remains to be demonstrated.

Vascular Protection Targets
Vascular protection is defined as an augmentation of endothelial function to prevent vascular smooth muscle cell proliferation, inflammation, thrombosis, and endothelial apoptosis. Although vascular protection is a distinct entity from neuroprotection, endothelial dysfunction may impact the ultimate degree of tissue damage in acute ischemic stroke. Moreover, recent observations suggest that endothelium and blood vessels not only provide metabolic sustenance for tissues but inductive signals for organogenesis.11,12 Therefore, mechanisms serving as targets for vascular protection should be evaluated in the context of ischemic stroke pathophysiology and can be separated into acute (hours), subacute (hours to days), and chronic (days to months) events, as depicted in Figure 1.

Acute
Vascular pathophysiology in the acute phase involves hemodynamic and metabolic changes, resulting in disruption of the blood–brain barrier (BBB) and dysregulation of vascular tone.13 The basal cerebrovascular tone, also referred to as myogenic tone, favors partial vasoconstriction and plays an important role in regulation of cerebrovascular blood flow in response to changes in perfusion pressure as well as to...
alterations in metabolic and endothelial factors by adjusting vessel caliber. Vascular smooth muscle and endothelium interactions contribute to the ability of the cerebrovasculature to respond to changes in perfusion pressure. Especially after ischemia, the function and integrity of cerebral arteries are critical to maintain cerebrovascular flow and tonus to minimize brain injury during reperfusion. Myogenic tone is diminished after 30 minutes of ischemia, and this impairment of the cerebrovasculature to maintain vascular tonus is associated with alterations in the actin cytoskeleton.

Interestingly, the thrombolytic agent tissue plasminogen activator, the only recommended treatment option to open an occluded artery, reduces reactivity of middle cerebral artery subjected to 2 hours of ischemia. Alterations in cerebrovascular resistance caused by impairment of vascular response may contribute to reperfusion injury and provide another explanation why use of thrombolytics more than 3 hours after the onset of stroke may increase the risk of bleeding.

Several factors including oxygen radicals as well as vasoactive factors such as NO, endothelin-1 (ET-1), vascular endothelial growth factor (VEGF), and angiopoietin I play important roles in regulation of vascular tone and structure in this acute phase of stroke. Reperfusion after partial or complete cerebral ischemia results in excessive production of oxygen radicals, which involves formation of hydrogen peroxide, hydroxy radicals, and predominantly superoxide. Superoxide generation peaks immediately after reperfusion and subsides during the next 2 hours. Vascular effects of superoxide are alterations in vascular response to CO2 and endothelium-dependent vasodilators such as acetylcholine, increased platelet aggregability, increased endothelial and BBB permeability, and disruption of endothelial cell

<table>
<thead>
<tr>
<th>Mediators</th>
<th>O2−, ET1</th>
<th>MMP-9, IL-1, TNFα, (MMP-2?)</th>
<th>Caspase, Bax, Trp53</th>
</tr>
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<tbody>
<tr>
<td>Protectors</td>
<td>NO, Angiopoietin 1, (VEGF),</td>
<td>VEGF, Angiopoietin 2, bFGF,</td>
<td>Bcl2, lap, SODs, VEGF</td>
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Time course of vascular damage after cerebral ischemia and reperfusion. In the acute phase, neutrophils adhere to the endothelium and, within hours, the BBB begins to leak. Mediators of vascular damage acutely include superoxide (O2−) and ET-1. Endogenously produced protectors of the vasculature include NO, angiopoietin 1, and potentially VEGF. In the subacute phase (hours), frank edema occurs, and if the injury is of sufficient magnitude, hemorrhagic transformation (HT) can occur. Mediators of this damage include MMP-9, IL-1, and potentially MMP-2. Protectors in the subacute phase include VEGF, angiopoietin 2, and bFGF. Chronically, the proapoptotic gene products caspases, Bax, and Trp53 predominate, and the antiapoptotic proteins Bcl2 and lap are protective. In addition, VEGF may promote angiogenesis, and superoxide dismutases (SODs) may protect against further oxidative damage. Eventually, the injured blood vessel may either promote recovery by angiogenesis or underapoptosis or atherosclerosis, depending on its size and function. PMN indicates polymorphonuclear leukocyte; RBC, red blood cell.
membranes.\textsuperscript{22} Nelson et al demonstrated that vasodilatation occurs during the early phases of reperfusion and parallels the time course of superoxide production in their experimental model.\textsuperscript{23} During this period, disruption of BBB permeability causes extravasation of albumin and other high-molecular weight compounds, which results in edema and increased intracranial pressure. In addition to its effects on BBB integrity and vascular tonus, superoxide anion reacts with NO to form peroxynitrite, which causes further tissue damage and is an important signaling mechanism that triggers inflammation and apoptosis in the subacute and chronic phases of ischemic stroke. A number of studies have reported that neutralizing free oxygen radicals by spin traps or scavenger enzymes such as superoxide dismutase or catalase prevents abnormal vasoreactivity as well as increased permeability of the BBB,\textsuperscript{23–26} and have provided evidence that limiting oxidant stress in the acute phase of ischemic stroke is critical for improving outcome. Administration of antioxidants such as α-lipoic acid and ginkgo biloba extract before induction of ischemia also provided neuroprotection and reduced infarct volume.\textsuperscript{27} A recent study reported that in addition to its antithrombotic actions, aspirin provides neuroprotection via its antioxidant effects in brain tissue subjected to hypoxia.\textsuperscript{28} However, the effect of antioxidants on cerebrovascular function and integrity and the potential therapeutic window in the setting of acute ischemia still remain unclear. Recent studies by Kontos et al demonstrated that glutamate, which accumulates in the extracellular space, stimulates superoxide production via activation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors.\textsuperscript{22} Thus, further studies evaluating blockade of AMPA receptors in prevention of excess superoxide production in the acute phase are needed.

There are a number of other vasoactive factors that affect endothelial function in the acute phase. For example, plasma and brain tissue levels of ET-1, a potent vasoconstrictor with mitogenic properties, are increased in patients with ischemic stroke as well as in animal models. In addition to its effects on vascular tonus, ET-1 increases BBB permeability.\textsuperscript{29–31} Local application of ET-1 has been shown to induce neuronal damage.\textsuperscript{30} A recent study by Matsuo et al demonstrated that administration of an endothelin type A receptor antagonist during reperfusion after transient middle cerebral artery occlusion significantly attenuated edema formation and mortality of animals after cerebral ischemia, providing evidence for the involvement of ET-1 in the pathophysiology of acute ischemic stroke.\textsuperscript{29}

Another important factor is VEGF, which is critical for angiogenesis and promotes endothelial integrity by stimulating NO production. However, VEGF increases BBB permeability in the acute phase after ischemic stroke.\textsuperscript{32,33} Therefore, VEGF administration in this phase may worsen neuronal damage. Although VEGF-induced NO production may be considered a means of improving endothelial integrity after ischemia, as discussed above, NO reacts strongly with superoxide to generate peroxynitrite that causes tissue damage. A recent study demonstrated that NO generated by neuronal NO synthase (NOS) during ischemia may be detrimental in addition to endothelial NO produced at reperfusion, causing damage via peroxynitrite formation.\textsuperscript{34}

Angiopoietin-1 is another growth factor involved in angiogenesis and regulation of BBB stability.\textsuperscript{35,36} Angiopoietin-1 levels decrease immediately after ischemia, and this coincides with increased BBB permeability. Therefore, it has been proposed to have a protective effect on BBB integrity. The balance between mediators of vascular damage and substances that protect the endothelium will determine whether the vasculature progresses onto a state of angiogenesis. At least in temporary ischemia limited to the striatum (30 minutes in the mouse), vascular protective factors give way to angiogenic factors within hours of reperfusion onset.\textsuperscript{37} In summary, vascular protection strategies in the acute phase should be based on approaches that prevent increased permeability of the BBB, including blocking superoxide formation as well as scavenging radicals that are produced during ischemia.

### Subacute

Starting with the subacute phase and continuing in the chronic phase of ischemic stroke, gene activation plays an important role in the pathophysiology of vascular dysfunction. In the subacute phase, a number of proinflammatory genes, including interleukin-1β (IL-1β), tumor necrosis factor α (TNF-α), and transcription factors such as hypoxia-inducible factor 1, nuclear factor κB, and interferon regulatory factor-1, are activated in response to the hypoxia, superoxide radical formation, and intracellular Ca\textsuperscript{2+} influx that occur during the acute phase.\textsuperscript{36–41} These proinflammatory products influence expression of adhesion proteins that are critical for integrity of vascular endothelium. For example, intercellular adhesion molecule 1, P-selectins, and E-selectins are upregulated.\textsuperscript{42–45} Blocking antibodies against these molecules have been shown to suppress complement activation, also implicated in furthering neurovascular injury after stroke.\textsuperscript{46–50} Adhesion proteins interact with neutrophils, allowing them to penetrate into the vasculature and brain tissue. Recent studies demonstrated that within 2 to 24 hours of ischemia, matrix metalloproteinase (MMP)-2 and MMP-9, which cause pathological remodeling by degrading the basal lamina and disrupting the integrity of endothelium, are also upregulated.\textsuperscript{51–56} Whether these proteins are stimulated by neutrophils or other proinflammatory gene products and to what extent they contribute to development of hemorrhagic complications of ischemia is still under investigation.

Inflammation could contribute to vascular injury by several mechanisms. First, increased leukocyte adhesion can cause microvascular obstruction, worsening ischemia.\textsuperscript{57} Second, in addition to increased expression of proinflammatory genes as discussed above, inducible NOS is upregulated in infiltrating neutrophils, causing production of toxic amounts of NO.\textsuperscript{58–60} Because inflammation is an important mechanism of vascular injury in the subacute phase with consequences such as endothelial cell death in the chronic phase, vascular protection strategies could be targeted to inhibition of neutrophil infiltration and toxic mediator production.

Gene activation involves not only expression of proteins that cause vascular injury but also induction of proteins that
typically serve a protective function. Studies on regulation of VEGF, angiopoietin, and basic fibroblast growth factor (bFGF) systems demonstrated that these proteins and respective receptors are also activated within 2 to 4 hours of ischemia.61–66 The roles of these proteins in vascular protection in acute ischemic stroke are not understood completely, but it is reasonable to speculate that these genes are activated as a feedback defense mechanism to restore endothelial function. The angiogenic properties of VEGF, angiopoietin 2, and FGF triggered studies evaluating potential use of these agents in therapeutic angiogenesis. A large number of studies provided evidence that VEGF induces re-endothelialization of blood vessels independent of its angiogenic effects, as discussed in detail in a recent review.4 In a model of neonatal hyperplasia where endothelium is intact, VEGF prevented intimal hyperplasia via an endothelial NO–dependent mechanism.67 Several studies demonstrated that VEGF-stimulated production of NO and prostaglandin I2 by endothelial cells could exert antiangiogenic effects as well as inhibit platelet aggregation and leukocyte adhesion to the endothelium via stimulation of cGMP and cAMP, respectively.68–70 Although these past studies provided evidence that VEGF could play an important role in vascular protection, pleiotropic and potentially protective effects of VEGF on the vasculature in the ischemic stroke setting should be evaluated in further detail and may offer an important protective strategy in the subacute and chronic phase of ischemic stroke.

Chronic
The mechanism of vascular changes in the chronic phase of ischemic stroke involves induction of genes that participate in the regulation of apoptosis as well as stimulation of angiogenic factors in endothelial cells. Programmed cell death is triggered by activation of cell surface receptors via several factors, including TNF-α, superoxide, and IL-1β, all of which are stimulated in the acute phase of ischemic stroke. In response to these stimuli, a cascade of proteolytic enzymes known as caspases and other proteins such as B-cell lymphoma-leukemia 2 (Bcl2)-associated X protein (Bax) and transformation related protein 53 (Trp53) as well as antiapoptotic proteins including Bcl2 and inhibitor of apoptosis protein (Iap), are activated.71–74 Therefore, inhibition of apoptotic gene expression and stimulation of antiapoptotic proteins may offer a vascular protection strategy. In addition to its angiogenic and antiproliferative effects as described above, VEGF also stimulates endothelial cell survival.75–77 It has been demonstrated that VEGF induces the antiapoptotic pathway through phosphatidylinositol 3-kinase, resulting in inhibition of endothelial apoptosis. These findings suggest that VEGF may offer additional protection in the chronic phase of ischemic stroke.

A Case for Intervention
It is unclear whether protection of the vasculature during acute cerebral ischemia will result in improved outcomes in human stroke patients. Currently, the only effective strategy for improving clinical outcomes after ischemic stroke is reperfusion therapy with thrombolysis.78–80 When an occluded artery is reopened, not only is the ischemic neuronal tissue spared, the vessel itself receives benefits associated with reperfusion. Neuroprotective strategies, although successful in experimental stroke models, have failed repeatedly to improve outcome in human stroke patients.5 Investigators have outlined a variety of reasons why all the clinical trials failed, but none include a role for vascular injury per se.81,82 It is possible that neuroprotective therapies have failed in humans because the damaged vasculature is unable to supply the necessary nutrients to the jeopardized tissue, nor is it able to deliver the requisite concentration of neuroprotectant to the tissue at risk. This is particularly important because the 2 main consequences of ischemic vascular damage in the brain are hemorrhagic transformation and ischemic cerebral edema.

On the other hand, vascular protection may not be the optimal therapeutic target in the acute phases of ischemic stroke. Some investigators have argued that vessel breakdown is necessary for initiation of angiogenesis and the recovery process after ischemic damage.37,83 Infiltration of circulating stem cells, which may release trophic factors necessary for neurogenesis, may depend on metalloproteinase activity to facilitate passage through the microvasculature.

Conclusions
Protection of the cerebral vasculature after ischemic stroke is a strategy that appears to be a logical approach to prevent edema and hemorrhage and enhance recovery. Enhancement of NO production and inhibition of VEGF, angiopoietin, angiotensin II, ET-1, the MMPs, and inflammatory mediators are all potential pharmacological targets for vascular protection. Although experimental evidence of the beneficial effects of vascular protection exists for both acute and chronic time points, only chronic vascular protection in human stroke patients has been proven as a therapeutic intervention. The optimal end points of clinical trials of vascular protection may include development of cerebral edema or hemorrhagic transformation along with overall neurologic function. Acute vascular protection needs to be explored in humans as a strategy to improve neurologic outcomes after an acute ischemic stroke.

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