Preconditioning the Human Brain
Proving the Principle in Subarachnoid Hemorrhage
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Organisms have developed complex endogenous defenses to counter environmental stress. The successful resistance to adversarial conditions, such as calorie or oxygen deprivation, has ensured that the strongest will survive. This lies at the root of preconditioning, that is, what is survived will strengthen; surviving mild forms of injury leads to tolerance of otherwise noxious injury. Preconditioning induces basic cellular survival mechanisms. Many different stimuli lead to preconditioning: drugs, ischemia, hypoxia, or hypothermia. In ischemic preconditioning exposing an organ to brief ischemia, induces temporary resistance to more severe ischemia, in the same or even a distant organ. The latter phenomenon is known as remote ischemic preconditioning. In this manner, for example, a limb is transiently made ischemic to protect the brain. Ischemic preconditioning has emerged as one of the most powerful anti-ischemic strategy in preclinical investigations.

In laboratory models, protection through preconditioning has been consistently demonstrated across multiple organ systems and in many different animal species, leaving little doubt about the existence of this phenomenon. A recent literature search (Medline September 2012), combining the terms preconditioning and brain, lists >1000 citations. This large body of laboratory evidence supports investigating whether, and to what an extent, protection through preconditioning can be reproduced in humans. Proof of concept work is needed, and with that the question arises, which clinical setting may be most suitable for preliminary clinical trials.

Preconditioning has been proposed as therapy for cerebral ischemia in several clinical settings of anticipated brain ischemia. These include carotid endarterectomy/stenting, coronary artery bypass graft surgery, and after subarachnoid hemorrhage (SAH). In the present review, we provide evidence that supports preconditioning for therapy of delayed cerebral ischemia (DCI) after SAH, arguing that proof of the ischemic preconditioning principle, may be most feasibly demonstrated in this setting.

Laboratory Models of Cerebral Preconditioning
Early studies of cerebral preconditioning included global ischemia models. Animals pretreated with 2 minutes of global brain ischemia showed marked protection against CA1 neuronal loss after 5 minutes global ischemia. Protection is also achieved in models of focal ischemia, simulating human stroke more closely. In a mouse model, brief occlusions of the middle cerebral artery decreased final infarct size after prolonged or permanent middle cerebral artery occlusion. Cross tolerance is also observed to occur between different organs. Short sublethal ischemia and reperfusion in 1 organ can induce ischemic tolerance in a distant organ. This is the remote ischemic preconditioning phenomenon. In a rat model of cardiac arrest, pretreatment with 15-minute bilateral forelimb ischemia resulted in a 54% increase in normal appearing CA1 neurons in comparison with an untreated control group. In a similar manner, remote limb preconditioning protects against focal cerebral ischemia. Rendering a limb transiently ischemic to protect the brain is of great clinical relevance, as this can be easily accomplished in humans.

Mechanisms of Protection in Ischemic Preconditioning
Ischemic preconditioning activates powerful endogenous anti-ischemia mechanisms that ultimately lead to enhanced survival. A very basic cellular stress response sets off multiple gene expression pathways that protect the brain. These result in the activation of several different mediators of which the following are of particular importance:

1. neurovascular protection
2. anti-inflammatory action
3. reduced excitotoxicity
4. metabolic protection.

Neurovascular Protection
Ischemic preconditioning has important protective effects on endothelial function and cerebral blood flow. In models of focal brain ischemia, ischemic preconditioning increased cerebral perfusion and penumbral blood flow, with improved recovery of cerebral blood flow in the postischemic period. These effects are mediated through the nitric oxide system. Increased immunoreactivity of inducible nitric oxide synthase (NOS) has been demonstrated in cerebral vasculature 24 hours after ischemic preconditioning. Blockade of endothelial NOS or knock-out mice, deficient in NOS, abolishes the

References

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protective effect of preconditioning. Nitric oxide (NO) is also involved in remote preconditioning. Increased NO and NOS activity was demonstrated in the rat CA1 hippocampal region after temporary clamping of the femoral artery. This was accompanied by a rise in serum NO and NOS, implicating possible humeral factors, released during reperfusion, as a potential mechanism of protection.

Anti-inflammatory Actions
Ischemic stroke is associated with cellular inflammation, which mediates secondary injury. Ischemia induces the migration of polymorphonuclear leucocytes and subsequently monocytes and results in the release of chemokines and cytokines with profound secondary vascular and neurotoxic effects. Ischemic preconditioning downregulates inflammatory markers and inflammation. In a rat model of focal ischemia, ischemic preconditioning reduced infarct size, which was associated with decreased postischemic mRNA expression of chemokines, cytokines, and multiple proinflammatory transcription factors. Preconditioning also reduces oxidative stress during focal cerebral ischemia.

Neuroprotection Through Reduced Excitotoxicity
Glutamate excitotoxicity is an important mediator of ischemic cell death. Glutamate antagonism has consistently shown to confer powerful neuroprotection in the laboratory. Preconditioning reduces glutamate excitotoxicity by decreasing release and promoting intracellular transport of glutamate and downregulating glial glutamate transporters. Conversely, higher levels of GABAergic neurotransmission inhibits excitotoxicity and is protective in focal models of brain ischemia. Ischemic preconditioning increases gamma-aminobutyric acid during brain ischemia through activation of glutamate decarboxylase.

Metabolic Protection
With preconditioning, cellular energy consumption is optimized and becomes more efficient, rendering the cell more resistant to hypoxia or ischemia. Energy metabolism is preserved through improved glycolysis, enhanced respiratory chain function, and stabilization of mitochondrial membranes. Preconditioning also involves a shift in the adenylate kinase equilibrium reaction in favor of adenosine triphosphate production, which is depleted during ischemia. This finding may have even more relevance for cerebral ischemia where the capacity for traditional mechanisms of energy maintenance, such as anaerobic metabolism, is limited.

Remote Ischemic Preconditioning
Special consideration should be given to mechanisms of remote ischemic preconditioning, as ischemic limb preconditioning has become the clinically most used method. In remote ischemic limb preconditioning an arm or leg is transiently made ischemic to achieve protection of a distant organ. How this transfer of tolerance occurs is not well understood but may involve humeral and neural pathways. Release of vasoactive substances, such as bradykinin, adenosine, and opioids from the ischemic limb, has been proposed to mediate this effect; neural pathways seem to be involved as well, as ganglion receptor blockers abolish the preconditioning response. Of additional interest is that measuring the degree of ischemia in the preconditioned organ can be readily achieved when an extremity is preconditioned. In this manner, muscle microdialysis during leg preconditioning has shown the successful induction of sublethal ischemia, with an increase in lactate/pyruvate ratio, without increases in glyceral, confirming preservation of cellular membranes.

Limitations of Laboratory Models of Ischemic Preconditioning
Laboratory models of preconditioning are limited in their translational value by the predictable onset of experimental stroke, the use of young un-stressed, disease-free animals, in which a preconditioning response may be more easily induced. Demonstrating whether a similar protective effect is even elicitable in humans and whether such a response is of therapeutic value will be much more difficult. The challenges consist of having to precondition before ischemia onset, in patients with multiple medical comorbidities, already under physiological stress and on medications that may modulate the preconditioning response.

Several clinical scenarios have been proposed to test the therapeutic preconditioning response in humans. These include preconditioning before carotid surgery/stenting, before coronary artery bypass surgery, and after SAH to prevent DCI. We believe SAH to be a particularly feasible clinical setting to evaluate the human preconditioning response. Ischemic preconditioning activates multiple pathways that have been invoked in the secondary injury after SAH. There is a high incidence of ischemic brain injury, which occurs over a relatively well-defined period, and patients with SAH tend to be younger and relatively free of concomitant disease.

SAH: Vasospasm and DCI
Although it is clear that initial subarachnoid bleeding is a major contributor of early brain injury, subsequent neurological deterioration from DCI remains an important cause of preventable morbidity and mortality. Stroke symptoms will develop in 30%, and 15% to 20% of patients will develop disabling stroke. Thick layering of blood around larger arteries is an important predictor of vasoconstriction, which results from a shift in the balance between the cerebral vasodilatory and the vasoconstrictive systems, as well as inflammation. Periarterial oxyhemoglobin causes the release of vasoactive substances that result in vasoconstriction. This effect is largely mediated by a downregulation of vasodilatory substances through deletion of NO and upregulation of vasoconstrictive substances, such as endothelin-1 and bilirubin oxidation products. There is loss of NOS in vessels with cerebral vasospasm and severe endothelial dysfunction. Strategies to augment NO and inhibit endothelin-1 have become therapeutic targets for improving outcome after SAH.

Contributing to these effects is an inflammatory reaction with infiltration of macrophages and granulocytes in the arterial wall and increased production of cytokines. C-reactive protein, interleukin-6, tumor necrosis factor,
intracellular adhesion molecule, and metalloproteinase 9 have been implicated as factors in the development of vasospasm and DCI after SAH. Cytokine inhibition, in animal models of SAH, has been associated with improved outcome.

DCI typically occurs between days 4 and 14 after the initial hemorrhage. Mechanisms leading to vasospasm and DCI are not fully understood and the 2 may occur independently of each other. Even though vasospasm leads to deficits in cerebral perfusion, the relationship of vasospasm to DCI is less well established. DCI may occur in the absence of clear spasm and those patients with the most severe spasm may not have DCI.

Prevention of DCI has emerged as a therapeutic goal in SAH. Multiple treatments have failed to improve outcome after SAH. In light of the very promising agents tested, the difficulties to develop a novel treatment for cerebral vasospasm are difficult to explain. A concern is the failure to affect multiple therapeutic molecular pathways in a very complex disease process. Targeting only a vasoactive pathway, such as the inhibition of vasoconstriction, may not be enough. This may have been a contributing reason for the failure of clazosentan, a powerful endothelin inhibitor, to improve neurological outcome after SAH, even though it effectively relieved vasospasm.

Preconditioning to Ameliorate Vasospasm and DCI

Preconditioning affects multiple molecular pathways implicated in mediating cerebral injury in SAH. In this manner, the enhancing effects of preconditioning on the NO system and anti-inflammatory effects are likely to induce neurovascular and endothelial protection. Inflammatory mediators, such as interleukin-6, tumor necrosis factor, intracellular adhesion molecule, matrix metalloproteinase 9, and C-reactive protein, have been linked to DCI and are downregulated through preconditioning. In addition, tissue is rendered more tolerant to ischemia through reduction of excitotoxicity and metabolic protection by enhancing mitochondrial function. Preconditioning also affects several other pathways that have been implicated in vasospasm and DCI. Activation of coagulation and reduced fibrinolysis, such as elevation of D-dimer and increased plasminogen activator inhibitor activity, is associated with DCI. Preclinical studies have shown that ischemic preconditioning upregulates fibrinolysis.

Preconditioning in Laboratory SAH Models

Most preclinical studies of preconditioning have included animal models of little translational value. Their purpose was to demonstrate that the preconditioning phenomenon can be induced in vivo. In an attempt to make laboratory investigations more clinically meaningful, several proof of principle studies have been conducted in experimental models of SAH. In a study of hypoxic preconditioning before the induction of SAH in mice, vasospasm and neurological deficits were prevented. The induction of NOS was shown to be a mediator of this protective effect. In a similar model, lipopolysaccharide preconditioning, before the induction of SAH in rats, was shown to improve vasospasm, reduce cerebral inflammatory cytokines, and prevent neurological deterioration.

A practical limitation for the clinical application of these studies is that preconditioning occurred before SAH. Nevertheless, these studies serve to demonstrate that innate protective systems are present and can be activated through preconditioning. In a model simulating human SAH more closely, resveratrol, a pharmaceutical preconditioning agent, whose effects have been compared with ischemic preconditioning, administered after induction of SAH in rats, led to a significant reduction in brain endothelin-1 levels, brain and serum lipid peroxidation levels, and a 43% increase in the luminal diameter of the basilar artery.

Potential Clinical Settings for Preconditioning: A Comparison

In a comparison with proposed clinical settings to examine the preconditioning response, several considerations may favor clinical testing in SAH. The relatively young and healthy population and frequent occurrence of study end points in SAH may make a demonstration of the preconditioning response more feasible. A common criticism of laboratory models of preconditioning is the use of young animals, relatively free of concomitant disease. Evidence suggests that the preconditioning response may be modulated by age and medications. In a clinical study assessing a potential preconditioning effect of transient ischemic attack before stroke, no protective effect of transient ischemic attack was found in patients >65 years. There is evidence that statins may modulate the preconditioning response. Given these deliberations, patients with SAH may be more suitable candidates for early studies of preconditioning than those patients typically undergoing carotid revascularization or coronary artery bypass. Patients with SAH tend to be younger, relatively free of comorbidities, and less likely to be taking multiple medications. In recent preconditioning studies, subjects with SAH were younger and relatively free of medical comorbidities (Table).

Selection of Study End Points for Preconditioning Trials in SAH

Clinical trials defining end points with higher event rates generally require fewer subjects. The end points for the 3 scenarios mentioned vary in their outcome event rate.

Table. Demographic and Clinical Characteristics of Subjects Enrolled in Clinical Preconditioning Trials

<table>
<thead>
<tr>
<th></th>
<th>SAH</th>
<th>CABG</th>
<th>CEA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, y</strong></td>
<td>53±12</td>
<td>67*</td>
<td>68*</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>42</td>
<td>61</td>
<td>62</td>
</tr>
<tr>
<td>Smoking, %</td>
<td>48</td>
<td>65</td>
<td>21</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>3</td>
<td>42</td>
<td>21</td>
</tr>
<tr>
<td>Ischemic heart disease, %</td>
<td>1</td>
<td>100</td>
<td>23</td>
</tr>
</tbody>
</table>

CABG indicates coronary artery bypass graft surgery; CEA, carotid endarterectomy; and SAH, subarachnoid hemorrhage. *SD not available.
Therapeutic interventions in coronary artery bypass graft surgery have typically examined postoperative neurocognitive decline, which occurs in 7% to 14% of patients. This would allow testing of interventions with moderate sample sizes (eg, in a recent study assessing the effect of reducing the cerebral, the lipid microembolism load during cardiopulmonary bypass a treatment effect was shown with >200 patients).66 Studies of carotid stenting or endarterectomy are complicated by significantly lower event rates. Investigators assessing preconditioning before carotid endarterectomy have concluded that unfeasibly large trials of up to 5000 patients would be needed to show even a minimal clinical effect.65 In SAH, DCI occurs with a relatively high incidence of 20% to 30%. End points of therapeutic trials in SAH include functional outcome scales, such as the Glasgow Outcome Score or modified Rankin Scale. Although there is a correlation with vasospasm and DCI, and 3-month functional outcome, treatments aimed at reducing vasospasm and DCI do not directly impact clinical outcomes (ie, the effects size of an intervention on vasospasm or DCI is not the same as for clinical outcome).66 The association with 3-month clinical outcome may be stronger for DCI than vasospasm.35 A recent analysis found that a prohibitively large sample size would be needed for clinical trials targeting vasospasm only. This may not be the case for treatments targeting DCI, which have a more direct impact on patient functional outcome. In The British Aneurysm Nimodipine Trial, neuroprotection with nimodipine led to a 35% reduction of cerebral infarction and 40% reduction in poor clinical outcome with a sample size of 554 subjects.68 The relatively high event rate of such end points in SAH would favor proof of concept trials for preconditioning in this setting. The event rate could be even further increased by enriching the target population and selecting patients at particularly high risk for DCI. This subgroup is easily identified before enrollment and includes those with thick layers of subarachnoid blood on admission brain computed tomography.67

Potential Pitfalls
None of the clinical settings considered here provides the clean ischemia model in which preconditioning has been tested in the laboratory, where cerebral ischemia is straightforwardly produced by direct vessel occlusion. This poorly replicates the diversity of human stroke, in which multiple factors, such as thromboembolism and hypoperfusion, act concomitantly. The pathophysiology of DCI remains complex and not fully understood. Neurological injury in SAH is multifactorial with several other mechanisms of injury potentially contributing. These include early brain injury from the initial hemorrhage, rebleeding, hydrocephalus, and metabolic derangements.69 This may obscure the detection of a preconditioning effect.

Similar concerns are applicable to coronary artery bypass graft surgery. Clinical stroke now rarely complicates coronary artery bypass surgery and the neurological injury manifests itself more subtly as postoperative neurocognitive decline, attributed to a combination of systemic inflammation, hypoperfusion at the time of bypass and embolism. Macroembolism may occur on occasion; however, microembolism from atherosclerotic arch debris and lipid embolism has been mostly implicated.56 Although such microembolism does lead to ischemic damage, the pathophysiology likely is quite different to that replicated in the laboratory. Additionally, cognitive decline continues postoperatively, which implicates factors not related to the bypass procedure. These include chronic progressive vascular disease and inflammatory brain damage, further complicating the use of coronary artery bypass graft surgery as an ischemic brain injury model for proof of concept studies.56,70 The ischemic injury during carotid stenting and endarterectomy probably replicates laboratory stroke most consistently in that stroke is caused by abrupt vessel occlusion. However, it occurs in the background of chronically ischemic tissue, induced by high-grade carotid disease. This in itself may act as a preconditioning stimulus and whether an additional preconditioning response above and beyond that can be induced remains uncertain.

Conclusions
We propose that SAH and DCI are specially well-suited clinical conditions for early proof of concept trials for the application of preconditioning. The use of preconditioning in these clinical settings is supported by a strong biological rationale for efficacy, favorable patient demographics, and practical aspects of clinical trial design that would make early proof of concept studies in this setting particularly feasible.

Disclosures
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Key Words: ischemic preconditioning ■ remote preconditioning ■ subarachnoid hemorrhage