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Neuroprotection in the Treatment of Acute Ischemic Stroke

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Abbreviations:

C - Celsius
HBOT – hyperbaric oxygen therapy
MI – myocardial infarction
RCT – randomized controlled trial
TLT – transcranial laser therapy
Abstract:
Neuroprotection remains one of the holy grails of acute ischemic stroke therapy. The ability to protect the ischemic brain from injury until reperfusion and then to protect the brain from reperfusion injury could theoretically improve freedom from disability among stroke survivors. This manuscript reviews the molecular and cellular pathophysiology of stroke and summarizes pharmacologic and other therapies that showed promise in pre-clinical testing as neuroprotection agents. However to date, no compelling efficacy data have been published regarding any pharmacologic or other therapies. Nonetheless the search for effective neuroprotection continues at stroke centers throughout the world.
Neuroprotection in acute ischemic stroke is the concept of administering therapy as rapidly as possible following the onset of symptoms in an effort to minimize cerebral infarction while the ischemic brain is awaiting reperfusion therapy. The term neuroprotection has also been used to describe the theoretical concept of decreasing additional neuronal injury that occurs upon reperfusion of the ischemic brain. The ideal neuroprotectant would be characterized by 1) a low risk of adverse effects and 2) be easily applicable in the pre-hospital setting so that the patient would receive benefit during pre-hospital transport, triage, imaging, and reperfusion therapy by fibrinolytic or endovascular methods. The medical literature is replete with well over 1000 published experimental reports and in excess of 100 clinical trials in this broad area of research (1,2). Despite numerous preclinical trials yielding dramatic benefit in animal models, no human data has yet demonstrated substantial efficacy to warrant a recommendation for use in the most recent American Heart Association / American Stroke Association Guidelines for the Early Management of Patients With Acute Ischemic Stroke (Table 1)(3).

**Physiologic Basis for Neuroprotection**

At a molecular level cerebral ischemia during stroke results in a depletion of cellular adenosine triphosphate at a rate higher than it can be synthesized. This results in lactic acidosis and, eventually, in loss of cellular homeostasis, especially with regard to cellular ionic concentrations. In neuronal tissue ionic imbalance results in release of neurotransmitters and inhibition of neurotransmitter re-uptake(4). Among the best-studied neurotransmitter pathways in stroke are those involving glutamate release. Glutamate triggers N-Methyl-D-aspartate (Figure 1) (5) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors. This, in turn, triggers calcium influx. High intracellular calcium concentrations result in activation of proteases and lipases resulting in cellular protein and membrane destruction (Figure 2)(5-7). Additionally reactive oxygen species are produced resulting in further cellular damage(8). Beyond direct molecular mechanisms of cell death and triggered apoptosis, cortical spreading depression may
occur, which is a phenomenon in which neuronal cells depolarize due to pathologic insult. The wavefront of depolarization affects contiguous cells and may result in increased metabolic activity among ischemic cells resulting in further cellular damage as well as a decrease in cerebral blood flow to the threatened penumbra (9). Interrupting or reversing this cascade of molecular and cellular events are the basis of potential neuroprotection therapies. An understanding of these events has resulted in the identification of potential targets to induce neuroprotection by either pharmacology or other treatment modalities.

**Pharmacologic Neuroprotection**

Over the last twenty to thirty years, numerous pharmacologic agents have been evaluated to interrupt the destructive pathophysiology of stroke and protect the brain. Therapeutic strategies have included minimizing the effects of excitatory amino acids, blunting transmembrane calcium fluxes, and limiting injury from inflammation, free radical damage, and intracellular enzymes. Many of the early studies were flawed by late administration of therapy within the four-to-six hour therapeutic window for brain reperfusion(2). In the majority of cases, treated subjects experienced no benefit and, in some cases, outcomes were worse than in control subjects(10). Agents utilized in these trials have included numerous different calcium channel blockers, but a meta-analysis of these agents concluded no evidence of benefit (11). Similar disappointing results have been evident in studies of the neurotransmitter precursor citicoline(12) as well as excitatory amino acid modulators(13), gamma-Aminobutyric acid agonists, magnesium(13) (14), and agents targeted at trapping free radicals (15), to name a few. The reasons for clinical failure of potentially favorable preclinical therapies are multiple. Beyond the methodological flaws in some clinical studies, it is important to recognize that experimentally induced stroke in animals does not mimic the heterogeneous nature of human stroke. Furthermore many animal studies do not take into account the aging human brain of many stroke patients(16). The stroke academic industry roundtable preclinical recommendations were
developed in order to provide guidance in designing preclinical trials of experimental therapeutics such that greater potential efficacy in clinical studies may be realized(17,18).

The only pharmacologic therapy to receive a recommendation for use in current guidelines is the hydroxymethylglutaryl co-enzyme A reductase inhibitor (i.e. statins)(3). Based on a small study of 89 patients randomized to continued statin therapy or statin withdrawal for 3 days after the onset of stroke, current recommendations are to continue statin therapy during the acute phase of stroke (class II A, level of evidence B). Patients evaluated in this small cohort demonstrated an increased odds ratio of death or dependency at 3 months if statins were withdrawn compared to those in whom statins were continued (19). A small, retrospective, single center study demonstrated that statin therapy may also decrease infarct volume by magnetic resonance imaging (20).

**Non-Pharmacologic Neuroprotection**

Alternative methods of neuroprotection include non-pharmacologic therapies aimed at altering the cellular apoptotic cascade of the ischemic penumbra. One such method is the administration of near-infrared laser therapy in which light energy at a wavelength of 808 nm is applied directly to the shaved skull of the patient in an effort to enhance brain recovery through a process called photobiostimulation (21). This technique is called transcranial laser therapy (TLT). The NEST-1 randomized controlled trial (RCT) provided pilot data supporting this hypothesis(22). This was followed by larger NEST-2 trial in which 660 patients were randomized to TLT or a sham procedure. The primary outcome of modified Rankin scale score at 90 days was not statistically significant between the two groups despite being numerically better in the treatment group(23). This led to the development of the even larger NEST-3 multi-center, RCT that will enroll 1000 patients(24). Hyperbaric oxygen therapy (HOBT) has also been studied. It is typically performed in a pressurized chamber in which oxygen is delivered to the patient at higher than normal atmospheres to maximize solubility of the gas within the patient's plasma and minimize the extraction of oxygen which is bound to circulating hemoglobin(25).
Potential complications of this therapy include barotrauma of the sinuses or middle ear, transient myopia, and, rarely, seizure activity. In fact, a small RCT of 33 acute stroke patients demonstrated worse outcomes in patients randomized to HBOT(26). However a more recent, small, open label study demonstrated improved National Institutes of Health Stroke Scale scores at 1 month in those patients who received HBOT(27). A Cochrane analysis of 11 RCT’s assessing HBOT for acute ischemic stroke concluded that the data published to date is insufficient to support incorporation of this therapy into treatment guidelines(28). The timing, duration, and frequency of HBOT after stroke might be important variables affecting treatment outcome. More research in this field is warranted.

There is a great deal of optimism among clinicians and research scientists for therapeutic hypothermia as a potent neuro-protectant. Its effect in preclinical animal models has been dramatic and is hypothesized to be due to 1) free radical production suppression, 2) limiting inflammatory mediators, 3) blunting the effect of excitatory amino acids, and 4) modification of ischemia-mediated calcium influx. The cooling blanket effect in the cellular milieu is thought to limit the apoptotic cascade so that neuronal cellular death is mitigated(29,30). Clinically, hypothermia has demonstrated benefit when used during circulatory arrest for major surgical procedures and is recommended in the routine treatment of comatose survivors of out of hospital cardiac arrest(31). There has also been enthusiasm for its use in the treatment of acute myocardial infarction (MI;AMI). In recent years, several trials have shown mixed results with some promise utilizing various cooling methods including iced saline infusion, endovascular cooling catheters, and iced peritoneal lavage (32-34). A recent meta-analysis of the AMI literature demonstrated evidence of reduction of infarct size in patients with anterior wall involvement, but no benefit in mortality, recurrent MI, or congestive heart failure(35).

To date, the majority of published clinical hypothermia data in stroke have focused on feasibility. Studies have assessed various cooling methods including the external application of pads or blankets, endovascular heat-exchange catheters, and cooling helmets (36-39). Three small
RCT’s in which stroke patients treated with thrombolysis were assigned to hypothermia or normothermia failed to demonstrate a significant treatment effect (40-42). Despite a widely-held hypothesis in the research community that therapeutic hypothermia holds great potential, a systematic review in 2009 prior to these 3 RCT’s showed no evidence of clinical benefit or harm. It was concluded that a clinically significant effect could not be ruled out, and large clinical trials were recommended (43). In the interim since that meta-analysis and those 3 RCT’s several studies have provided renewed enthusiasm and some concern for neuroprotection with hypothermia. A prospective cohort study demonstrated that therapeutic hypothermia resulted in less cerebral edema (P=0.001), less hemorrhagic transformation (P=0.016), and better outcomes (P=0.017) in a small non-randomized population (44). The ICTUS-2 trial planned to randomize 1600 ischemic stroke patients to standard care or hypothermia. It was stopped early after 120 of 1600 patients were enrolled due to an increased incidence of pneumonia in the cooling group (45). The COOLIST trial randomized awake, stroke patients to surface cooling with a target temperature of 35 Celsius (C), 34.5 C, or 34 C. The trial was stopped early due to slow enrollment. However of the 22 patients enrolled, 8 who were randomized to cooling developed pneumonia. None of the controls developed pneumonia. The trial demonstrated a potential shortcoming of surface cooling in awake patients i.e. hypothermia may be limited to 34.5 C (46). It is hoped that more robust data will come from the EuroHYP-1 RCT, which will assign 1500 stroke patients to either hypothermia or normothermia. The hypothermia target will be between 34C and 35C for 24 hours achieved with either surface or endovascular cooling (47). Nonetheless, the optimal timing, duration, and goal temperature of hypothermia for stroke remain unknown and warrant further study.

**Summary**

Neuroprotection remains one of the holy grails of acute ischemic stroke therapy. The ability to protect the ischemic brain from injury until reperfusion and then to protect the brain
from reperfusion injury could theoretically improve freedom from disability among stroke survivors. Molecular targets for neuroprotection have been identified. However to date, no compelling efficacy data have been published regarding any pharmacologic or other therapies. Nonetheless the search for effective neuroprotection continues at stroke centers throughout the world.
References


Table 1

**AHA/ ASA Recommendations for Neuroprotection During Stroke**


<table>
<thead>
<tr>
<th>Recommendation</th>
<th>Class</th>
<th>Level of Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among patients already taking statins at the time of onset of ischemic stroke, continuation of statin therapy during the acute period is reasonable</td>
<td>IIa</td>
<td>B</td>
</tr>
<tr>
<td>The utility of induced hypothermia for the treatment of patients with ischemic stroke is not well established, and further trials are recommended</td>
<td>IIb</td>
<td>B</td>
</tr>
<tr>
<td>At present, transcranial near-infrared laser therapy is not well established for the treatment of acute ischemic stroke</td>
<td>IIb</td>
<td>B</td>
</tr>
<tr>
<td>At present, no pharmacological agents with putative neuroprotective actions have demonstrated efficacy in improving outcomes after ischemic stroke, and therefore, other neuroprotective agents are not recommended</td>
<td>III</td>
<td>A</td>
</tr>
<tr>
<td>Data on the utility of hyperbaric oxygen are inconclusive, and some data imply that the intervention may be harmful. Thus, with the exception of stroke secondary to air embolization, this intervention is not recommended for treatment of patients with acute ischemic stroke</td>
<td>III</td>
<td>B</td>
</tr>
</tbody>
</table>
Figure 1. The role of glutamate and the NMDA receptor in the pathophysiology of stroke

Distinct subpopulations of the NMDA receptor (NMDAR) mediate neuronal death and survival. (a) Under normal conditions, synaptic activity maintains neuronal survival via activation of the synaptic NMDAR. This pro-survival effect is dependent on the calcium influx through the receptors. (b) During cerebral ischemia, excessive release of glutamate into the synapses and extrasynaptic sites causes global stimulation of NMDAR at both locations. The C-terminal domain of the GluN2B subunit acts as a major hub for recruiting death-signaling proteins, which in turn is activated by calcium influx through the receptors to induce neuronal death.

Figure 2. Cellular and molecular pathophysiology of stroke
The major cellular and molecular events that occur after cerebral ischemia and reperfusion are outlined in this diagram. Potential neuroprotection therapies aim to interrupt or reverse these events. AMPA, α-amino-3-hydroxy-5-methylisoxasole-4-propionic acid; NMDA, N-methyl-D-aspartate; NO, nitric oxide; iNOS, inducible nitric oxide synthase; IP3, inositol 1,4,5-triphosphate; COX2, cyclooxygenase-2.