Neuroprotective strategies and the underlying molecular basis of cerebrovascular stroke

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Stroke is a leading cause of disability in the US. Although there has been significant progress in the area of medical and surgical thrombolytic technologies, neuroprotective agents to prevent secondary cerebral injury and to minimize disability remain limited. Only limited success has been reported in preclinical and clinical trials evaluating a variety of compounds. In this review, the authors discuss the most up-to-date information regarding the underlying molecular biology of stroke as well as strategies that aim to mitigate this complex signaling cascade. Results of historical research trials involving *N*-methyl-D-aspartate and α -amino-3-hydroxy-5-methyl-4-isoxazole propionate receptor antagonists, clomethiazole, antioxidants, citicoline, nitric oxide, and immune regulators have laid the groundwork for current progress. In addition, more recent studies involving therapeutic hypothermia, magnesium, albumin, glyburide, uric acid, and a variety of other treatments have provided more options. The use of neuroprotective agents in combination or with existing thrombolytic treatments may be one of many exciting areas of further development. Although past trials of neuroprotective agents in ischemic stroke have been limited, significant insights into mechanisms of stroke, animal models, and trial design have incrementally improved approaches for future therapies.

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KEY WORDS neuroprotection; stroke; cerebrovascular accident; intraparenchymal hemorrhage; intracerebral hemorrhage

CEREBROVASCULAR ischemia, or stroke, affects up to 800,000 US individuals yearly and is a leading cause of disability, costing \$34 billion per year in health care expenses, disability, and lost productivity.¹⁰⁹ Although there have been significant advances in intravenous tissue plasminogen activator (TPA) and mechanical thrombectomy approaches, there remains a significant need for additional neuroprotective and neuroregenerative therapies.^{74,109,136} Neuroprotection involves strategies to interrupt the cascade of cell injury leading to infarction.⁵¹ Approximately 85% of stroke is ischemic and 15% of stroke is hemorrhagic; however, the use of neuroprotective agents may be beneficial and specific to both types.¹⁰⁹

The number of studies involving neuroprotective treatments has increased exponentially since elucidation of the molecular mechanisms of stroke in the 1970s. Preclinical and clinical studies have further improved the pathophysi-

ological understanding of ischemic stroke and helped focus development of further approaches.⁴⁸ However, great challenges remain in identifying agents with significant therapeutic efficacy. Currently, there is no US Food and Drug Administration–approved agent for neuroprotection after ischemic stroke. Despite this hurdle, ongoing studies are investigating various approaches. The Internet Stroke Center (<http://www.strokecenter.org/>) describes 3867 active or completed trials, including 60 trials regarding neuroprotective agents, with many that are actively recruiting patients (Table 1). Similarly, clinicaltrials.gov reports 58 studies evaluating neuroprotection that are actively recruiting, including for ischemic stroke and other neurological pathologies. Various state and national stroke registries have also been key in organizing collaborations between various hospitals and serving as a source of information for neuroprotection clinical trials. The purpose

ABBREVIATIONS AMPA = α -amino-3-hydroxy-5-methyl-4-isoxazole propionate; ATP = adenosine 5'-triphosphate; GABA = γ -aminobutyric acid; IL = interleukin; mRS = modified Rankin Scale; NIHSS = National Institutes of Health Stroke Scale; NMDA = *N*-methyl-D-aspartate; RCT = randomized controlled trial; SUR1-TRPM4 = sulfonylurea receptor 1–transient receptor potential melastatin 4; TPA = tissue plasminogen activator.

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TABLE 1. Current trials actively enrolling patients for neuroprotective treatment strategies

Trial Name	Trial Abbreviation	Trial No.
Study to Assess the Safety, Pharmacokinetics, and Pharmacodynamics of DS-1040b in Subjects With Acute Ischemic Stroke	ASSENT	NCT02586233
Administration of Statin On Acute Ischemic Stroke Patient Trial	ASSORT	NCT02549846
Cooling Plus Best Medical Treatment Versus Best Medical Treatment Alone for Acute Ischaemic Stroke	EuroHYP-1	NCT01833312
Effects of Cerebral Protection With Filters vs. Flow Reversal on Cerebral Embolization After Carotid Artery Stenting		NCT01414387
Effects of Surface Cooling On Stroke Outcome Trial	COOL	NCT02176993
Efficacy and Safety Study of GM602 in Patients With Acute Middle Cerebral Artery Ischemic Stroke Within 18 Hours	GMAIS	NCT01221246
Field Randomization of NA-1 Therapy in Early Responders	FRONTIER	NCT02315443
Hydroxyurea to Prevent Brain Injury in Sickle Cell Disease	HUPrevent	NCT01389024
Implant for Augmentation of Cerebral Blood Flow Trial, Effectiveness and Safety in a 24-Hour Window	ImpACT-24	NCT00826059
Intensive Insulin Therapy With Tight Glycemic Control to Improve Outcomes After Endovascular Therapy for Acute Ischemic Stroke		NCT02054429
Intra-arterial Bone-Marrow Mononuclear Cells Infusion for Acute Ischemic Stroke		NCT02178657
Neuroprotection of Pioglitazone in Acute Ischemic Stroke		NCT02195791
Neuroprotection with Statin Therapy for Acute Recovery Trial (Neu-START): A Phase II safety and pilot efficacy study of lovastatin for the treatment of acute ischemic stroke [SPOTRIAS]	Neu-START II/ SPOTRIAS	NCT00243880
Neuroprotective Effect of Autologous Cord Blood Combined With Therapeutic Hypothermia Following Neonatal Encephalopathy		NCT02551003
Neuroprotective Goal Directed Hemodynamic Optimization in Post-Cardiac Arrest Patients: a Randomized Controlled Trial (the NEUROPROTECT Post-CA Trial)	NEUROPROTECT	NCT02541591
Remote Ischaemic Conditioning After Stroke Trial	ReCAST-2	NCT02779712
Remote Ischemic Conditioning in Acute Brain Infarction Study	RESCUE-BRAIN	NCT02189928
Safety Evaluation of 3K3A-APC in Ischemic Stroke	RHAPSODY	NCT02222714
Stroke and Tocotrienol: Unique Role in Neuroprotection	SATURN	NCT02263924
Study of Compound Edaravone Injection for Treatment of Acute Ischemic Stroke		NCT02430350
Subcutaneous Interleukin-1 Receptor Antagonist (SC IL-1RA) in Stroke Study		ISRCTN74236229
The Effect of GLP-1 Receptor Agonist on Cerebral Blood Flow Velocity in Non-Stroke Volunteers	EGRABINS1	NCT02838589
The Effect of GLP-1 Receptor Agonist on Cerebral Blood Flow Velocity in Stroke	EGRABIS1	NCT02829502
The Efficacy of Citalopram Treatment in Acute Stroke	TALOS	NCT01937182
The RIGHT Trial: Rapid Intervention with Glyceryl Trinitrate (GTN) in Hypertensive Stroke Trial	RIGHT	ISRCTN26986053
Safety Evaluation of 3K3A-APC in Ischemic Stroke	RHAPSODY	NCT02222714
The Safety and Efficacy Study of High-Dose Atorvastatin After Thrombolytic Treatment in Acute Ischemic Stroke	SEATIS	NCT02452502
Stroke Hyperglycemia Insulin Network Effort (SHINE) Trial	SHINE	NCT01369069

of this review is to discuss recent studies regarding the molecular mechanism of stroke, clinical trial findings, and potential future approaches.

Pathophysiology of Ischemic Stroke

A complex, coordinated, and interrelated cascade of molecular events follows brain ischemia and infarction. Ischemia is defined as a reduction in blood flow sufficient to cause an alteration of normal cellular function; hypoxia is defined as a reduction of tissue oxygen to levels insufficient to maintain homeostasis. Initial events after ischemia result in necrosis of core infarcted tissue with reduced blood flow, maintained metabolism, and reduced function of adjacent penumbra tissue.¹⁶ After disruption of adenosine 5'-triphosphate (ATP) generation and the Na⁺/

K⁺ transporter, cellular depolarization allows Ca²⁺ influx, resulting in activation of the intrinsic apoptosis pathway and cell death.⁸⁶ Glutamate accumulation in the extracellular space results in activation of *N*-methyl-D-aspartate (NMDA), α -amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA), and kainate glutamate receptors, which mediate Ca²⁺ influx.⁸⁷

Increased oxidative stress occurs during free radical formation by superoxide, hydroxyl radical, nitric oxide, and peroxynitrite, with reduction of energy-dependent scavenger enzymes during stroke.⁹⁴ Free radical generation also occurs from arachidonic acid metabolism,⁵⁹ infiltrating peripheral leukocytes,¹³⁸ and upregulation of inducible nitric oxide synthase by microglia and monocytes,¹⁵⁶ all of which are seen during acute stroke. Free radicals and Ca²⁺

induce inflammatory cytokines (e.g., interleukin [IL]–1, IL–6, tumor necrosis factor– α) and chemokines (e.g., IL–8, MCP–1) as well as endothelial cell adhesion molecules (e.g., selectins, ICAM–1, VCAM–1) and proinflammatory genes.^{32,70,156} Free radicals also result in lipid peroxidation, induction of apoptosis, and production of 4-hydroxynonenal, which covalently modifies the Na⁺/K⁺ ATPase, glucose, and glutamate transporters to impair function.¹⁰¹

Distinct cell populations also play important roles in ischemic response. Critical to the process of inflammation is the role of microglia within the ischemic penumbra, which produce proinflammatory cytokines, toxic metabolites, and enzymes.^{29,115} Astrocytes also produce proinflammatory cytokines and neuroprotective factors (e.g., erythropoietin, transforming growth factor– β , metallothionein–2).¹⁰⁴ Endothelial changes can also result in blood-brain barrier dysfunction and increased inflammatory response.²⁸ Infiltration of circulating leukocytes, including polymorphonuclear leukocytes and T cells, impairs recovery after ischemic stroke.^{12,46,67,100} Increased cerebral edema after ischemic stroke also plays a role in poorer patient prognosis.¹³⁷ Involvement of the sodium-hydrogen antiporter family members, sulfonylurea receptor 1–transient receptor potential melastatin 4 (SUR1-TRPM4) cation channel, and aquaporin–4 is particularly important in mediating increased cerebral edema after ischemic stroke. Hemorrhagic stroke also differs from ischemic stroke with the release of cytotoxic hemoglobin, upregulation of the acute phase proteins haptoglobin and hemopexin, and increased oxidative stress (Fig. 1).¹⁰

Key Historical Clinical Findings

Although many preclinical studies have helped to improve understanding of ischemic stroke (Table 2), the studies often differed substantially in terms of animal factors, treatment conditions, models of ischemia, timing of treatment, and outcome assessment. From 1995 to 2015, 430 potential drugs for stroke were evaluated worldwide, but only 19 (4%) successfully reached the market.¹⁹ Of the remaining candidates, approximately 300 were discontinued, 70 continued preclinical evaluation, and 40 were in clinical trials. Among drugs currently in clinical use, two-thirds are used for thrombus dissolution (e.g., plasminogen activators, antithrombotic agents, platelet aggregation agents) and one-third for neuroprotection. Other neuroprotective, nonthrombolytic treatments that are used outside of the US include Ginkgo Mihan, *Lumbricus rubellus* extract, fasudil, Cerebrolysin, citicoline, kallidinogenase, and edaravone.¹⁹

Nimodipine: A Calcium Channel Inhibitor

Calcium channel inhibition with nimodipine, a 1,4-dihydropyridine calcium channel antagonist and cerebral vasodilator, has been evaluated in a variety of preclinical⁶⁴ and clinical randomized controlled trials (RCTs) in patients with stroke. Clinical evaluation involved the American Nimodipine Study,⁷ the TRUST Study,¹⁴⁶ and the VENUS trial,⁶³ among others.^{47,73,149} Two meta-analyses evaluating patients from both large and smaller ischemic stroke trials that assessed > 7500 patients in total failed to

find an overall benefit for patients.^{65,107} Subgroup analysis did show a benefit in neurological function in patients treated within 12 hours, but patients who received intravenous nimodipine showed worse outcomes. Criticism of these trials included treatment at 24–48 hours after stroke, which was beyond the therapeutic window, as well as inclusion criteria, outcome measures, and limited preclinical support for this treatment.

Glutamate Receptor Inhibitors

Among the agents assessed for an ability to reduce glutamate excitotoxicity, NMDA and AMPA receptor inhibitors have been evaluated in preclinical and clinical trials but have shown limited therapeutic benefit.^{3,25,57,127,147,151} These results suggest that non–NMDA-mediated mechanisms are also important in neuronal excitotoxicity.⁶⁹ Some non–NMDA excitotoxic candidates include acid-sensing ion channel 1a,¹¹⁵ proton-sensitive cation channels,⁸³ and transient receptor potential channels.¹⁵⁹

Glutamate, the main excitatory neurotransmitter, can result in excitotoxic neural injury during ischemia mediated by Ca²⁺ cell influx in a variety of neurological disorders.¹⁰² Preclinical trials extensively evaluated the noncompetitive NMDA antagonists MK–801/dizocilpine, dextromethorphan, and aptiganel.^{51,105} Phase I dose-escalation trials of dextromethorphan trials involving clinical treatment within 48 hours of stroke showed significant side effects, including hallucinations, agitation, hypotension, and stupor or apnea at high doses.² MK–801 was also associated with significant neuropsychological adverse effects.¹¹⁶ A Phase II/III RCT in 628 patients treated within 6 hours of stroke with high- or low-dose aptiganel did not achieve the primary outcome of modified Rankin Scale (mRS) score at 3 months.³ Furthermore, a higher 4-month mortality rate was seen in the aptiganel group (26% vs 19%), and the study was aborted. The competitive NMDA antagonist CGS–19755/selfotel was evaluated preclinically and clinically,¹⁰⁶ but similar neuropsychological adverse events were reported, including agitation, hallucination, paranoia, and delirium.⁵⁷ Two Phase III RCTs involving 567 subjects that evaluated selfotel administered within 6 hours of stroke were suspended because there seemed to be a higher mortality rate in the selfotel-treated group.^{24,25}

Glycine antagonism of the NMDA receptor by GV150526/gavestinel resulted in reduction of infarct size in preclinical models.¹⁶ Two Phase III RCTs, namely the Glycine Antagonist in Neuroprotection (GAIN) American Trial and the GAIN International Trial, of 1646 and 1804 subjects, respectively, were conducted.⁸¹ Treatment was given within 6 hours of ischemic stroke, but neither trial showed an improvement in the primary outcome of Barthel Index at 3 months. A subgroup analysis of 571 patients with hemorrhagic stroke also failed to demonstrate any benefit from treatment with gavestinel.⁵⁸

AMPA receptor antagonists, such as ZK200755 and YM872/zonampanel, have been evaluated in preclinical and clinical models, with apparently poor results.^{38,51,142} In a Phase II RCT that was ultimately suspended after 61 patients were enrolled, ZK200755 showed worsening National Institutes of Health Stroke Scale (NIHSS) score

FIG. 1. Pathophysiology of ischemic stroke and neuroprotective strategies. The basic underlying pathology of ischemic stroke involves excitotoxicity and failure of cellular homeostatic functions, resulting in upregulation of calcium and free radicals. The end result involves cellular demise, maladaptive genetic expression, altered proteolysis, lipid peroxidation, recruitment of immune cells, and further inflammatory cascades. A variety of approaches (shown in *red text*) have attempted to target these various aspects. AA = arachidonic acid; CaMK = calcium/calmodulin-dependent protein kinase; COX = cyclooxygenase; H_2O_2 = hydrogen peroxide; KA = kainate; LOX = lipoxygenase; NO = nitric oxide; NOS = nitric oxide synthase; OH = hydroxide ion; OONO = peroxynitrite; PLA2 = phospholipase A2; SOD = superoxide dismutase; SSRI = selective serotonin-reuptake inhibitor.

and elevated S-100B biomarker.³⁸ The AMPA Receptor Antagonist Treatment in Ischemic Stroke MRI Trial (ARTIST) and related ARTIST+ trial evaluated YM872/zonampanel, but no peer-reviewed reports of the trial results were published.⁵¹ A recent meta-analysis of 7731 patients and 34 trials involving calcium channel antagonists

also failed to show any benefit with nimodipine and in fact demonstrated worse outcome at higher doses.¹⁶⁰

γ -Aminobutyric Acid Agonists

γ -Aminobutyric acid (GABA) agonists have widely been studied for their role in the major inhibitory neu-

TABLE 2. Historical treatments in neuroprotection for ischemic stroke

Treatment	Mechanism	References
Calcium channel antagonist		
Nimodipine	1,4-dihydropyridine calcium channel antagonist & cerebral vasodilator	American Nimodipine Study Group 1992, ⁷ Trust Study Group 1990, ¹⁴⁶ Horn et al. 2001, ⁶³ Gelmers et al. 1988, ⁴⁷ Horn & Limburg 2000, ⁶⁵ Kaste et al. 1994, ⁷³ Mohr et al. 1994, ¹⁰⁷ Wahlgren et al. 1994 ¹⁴⁹
Glutamate antagonists		
MK-801/dizocilpine	NMDA antagonists, reduce glutamate excitotoxicity	Olney 1994 ¹¹⁷
Dextromethorphan		Albers et al. 1995 ²
Aptiganel		Albers et al. 2001 ³
CGS-19755/selfotel		Miyabe et al. 1997, ¹⁰⁶ Davis et al. 1997, ²⁴ Davis et al. 2000 ²⁵
GV150526/gavestinel	Glycine antagonist at the NMDA receptor, reduces glutamate excitotoxicity	Bordi et al. 1997, ¹⁶ Sacco et al. 2001, ¹²⁴ Lees et al. 2000, ⁸¹ Haley et al. 2005 ⁵⁸
ZK200755	AMPA receptor antagonists	Elting et al. 2002 ³⁸
YM872/zonampanel		Ginsberg 2008 ⁵¹
Magnesium	NMDA receptor voltage-dependent modulator that reduces depolarization-induced excitotoxicity	Yang et al. 2000, ¹⁵⁸ Westermaier et al. 2005, ¹⁵⁵ Meloni et al. 2006, ¹⁰³ Muir et al. 2004 ¹¹⁰
GABA agonists		
Clomethiazole	GABA agonists	Sydserrff et al. 1995, ¹⁴⁰ Marshall et al. 1999, ⁹⁸ Wahlgren et al. 1999 ¹⁵⁰
Diazepam		Lodder et al. 2006 ⁹³
Antioxidants		
NXY-059 (disodium 4-[(tert-butylimino)methyl]benzene-1,3-disulfonate N-oxide)	Antioxidant agent	Kuroda et al. 1999, ⁷⁹ Shuaib et al. 2007, ¹³⁴ Ginsberg 2007 ⁵⁰
Tirilazad mesylate	Lipid peroxidation inhibitor	Sena et al. 2007, ¹²⁹ Randomized Trial of Tirilazad Mesylate in Patients with Acute Stroke (RANTTAS) 1996, ¹²⁰ Tirilazad International Steering Committee 2000 ¹⁴⁴
Edaravone/MCI-186/3-methyl-1-phenyl-2-pyrazolin-5-one	Lipid peroxidation inhibitor & free radical scavenger	Amemiya et al. 2005, ⁶ Edaravone Acute Infarction Study Group 2003, ³⁵ Feng et al. 2011, ⁴¹ Shinohara et al. 2009 ¹³³
Ebselen/2-phenyl-1,2-benzisoselenazol-3(2H)-one	Selenium-based compound w/ glutathione peroxidase-like activity	Namura et al. 2001 ¹¹³
Citicoline	Cytidine 5-diphosphocholine mimicker that incorporates into neuronal membranes, causing increased phospholipid synthesis & decreased degradation, increased neurotransmitter levels, improved ATPase function, & decreased phospholipase A2 activity & reactive oxygen species generation	Scandinavian Stroke Study Group 1988, ¹²⁶ Italian Acute Stroke Study Group 1988, ⁷² Amsterdam Stroke Study 1992, ⁵⁵ Goslinga et al. 1992, ⁵⁵ Argentino et al. 1989, ⁹ Aichner et al. 1998 ¹
Hemodilution	Reduces excitotoxicity & metabolic demand	Kuroda et al. 1999, ⁷⁹ Shuaib et al. 2007, ¹³⁴ Ginsberg 2007 ⁵⁰
Nitric oxide mediators		
Lubeluzole	4-difluoro benzothiazole; thought to downregulate glutamate-activated nitric oxide	De Ryck et al. 2000, ²⁶ Grotta 1997, ⁵⁶ Diener 1998, ³⁰ Diener et al. 2000, ³¹ Gandolfo et al. 2002 ⁴⁵
Fasudil	Rho kinase inhibitor; regulates proliferation, apoptosis, & cell motility; endothelial nitric oxide mediator	Shibuya et al. 2005, ¹³² Kubo et al. 2008, ⁷⁸ Liao et al. 2007 ⁸⁴
Immunomodulator		
Enlimomab	Intercellular adhesion molecule-1 monoclonal antibody inhibitor that reduces leukocyte adhesion	Zhang et al. 1995, ¹⁶¹ Enlimomab Acute Stroke Trial Investigators 2001, ³⁹ Krams et al. 2003 ⁷⁷

rotransmitter pathways. One of the most extensively studied preclinical models of neuroprotection, clomethiazole, reduced ischemic territory after stroke by 58% in rats¹⁴⁰ and 32% in small primates.⁹⁸ The Clomethiazole Acute

Stroke Study (CLASS) evaluated 1360 patients randomly assigned to receive clomethiazole or placebo within 12 hours of stroke but did not find a difference in functional independence at 90 days.¹⁵⁰ The subsequent randomized

CLASS-I trial evaluated 1198 patients with ischemic stroke but did not achieve a difference in primary outcome of a Barthel Index ≥ 60 at 90 days. Diazepam, another GABA agonist, was evaluated in a trial of 880 patients who had stroke but also failed to achieve a difference in primary outcome, which was independence on the RS at 3 months.⁹³ A recent meta-analysis of 5 trials of GABA receptor agonists that included 2838 patients did not show a significant difference in outcome.⁸⁹

Magnesium as Treatment for Stroke

Magnesium has been implicated for its importance in regulating the NMDA receptor by modulating voltage-dependent channel depolarization excitotoxicity; it is used clinically for treatment of eclampsia.³³ Preclinical models of magnesium showed reduction in rat infarct volumes in several trials,^{155,158} but these studies showed varying effects and confounders.¹⁰³ Magnesium administration within 12 hours of stroke was evaluated in the clinical Intravenous Magnesium Efficacy in Stroke (IMAGES) Trial; the investigators found no difference in the rates of death or disability at 90 days in 2589 patients with acute stroke.¹⁰⁹

Antioxidant Agents

A variety of antioxidant agents have been evaluated in ischemic stroke to reduce secondary damage. In preclinical trials of ischemia, the antioxidant NXY-059 (disodium 4-[(tert-butylimino)methyl] benzene-1,3-disulfonate N-oxide) showed reduction of infarction by 77% in rodents⁷⁹ and 51% in primates.⁹⁹ NXY-059 was evaluated in the Stroke–Acute Ischemic NXY Treatment I (SAINT I) trial with 1722 patients⁸² and the SAINT II trial with 3306 patients.¹³⁴ The SAINT I trial showed that NXY-059 improved the distribution of mRS scores compared with placebo, but did not affect the NIHSS scores or Barthel Index in patients. In addition, this clinical effect was small. The SAINT II trial did not show a difference between NXY-059 and placebo. The trial demonstrated that the limited solubility and blood-brain barrier penetrance of NXY-059 were problematic. In addition, there was a long mean time of > 6 hours until potential treatment. Overall, NXY-059 seemed to have reduced antioxidant potential compared with other compounds, such as vitamin E.⁵⁰

Tirilazad mesylate is a lipid peroxidation inhibitor that has been evaluated in preclinical trials involving a variety of neurological injuries, including stroke, where it reduced infarct volume by 29%.¹²⁹ Nevertheless, clinical trials have not demonstrated the same success. In fact, a meta-analysis of 6 trials of tirilazad in 1757 patients showed an increased risk of death or disability by 20%.¹⁴⁴ The Randomized Trial of Tirilazad mesylate in patients with Acute Stroke (RANTTAS) was terminated early because an interim analysis of 556 patients showed no effect on outcome.¹²⁰

Ebselen/2-phenyl-1,2-benzisoselenazol-3(2H)-one is a selenium compound with glutathione peroxidase–like activity that has shown reduction in infarct size in rodent models.¹¹² A clinical trial of 302 patients showed a significant difference in primary outcome at 1 month but not at 3 months.¹⁵⁷ Edaravone/MCI-186/3-methyl-1-phenyl-2-pyrazolin-5-one is a lipid peroxidation inhibitor and free radical scavenger that has also shown benefit in preclinical

models, with reduction in infarct volume by 30%.⁶ Patients in a Phase II clinical trial of edaravone did show improvement of mRS score at 3 months,³⁵ and a meta-analysis of 3 trials (496 patients) showed overall improvement in the proportion of neurological improvement in patients treated with edaravone compared with control subjects (risk ratio 1.99, 95% CI 1.6–2.49).⁴¹ However, the most recent RCT of edaravone in 401 patients failed to show a difference in mRS score < 1 between edaravone and ozagrel (an antiplatelet thromboxane A2 synthesis inhibitor) (57.1% vs 50.3%).¹³³ Nevertheless, the culmination of these results was the basis for approval in 2001 of this treatment for acute stroke in Japan.

Citicoline, another class of compound resembling cytidine 5-diphosphocholine, has been evaluated extensively. It is incorporated into neuronal membranes where it causes increased phospholipid (e.g., phosphatidylcholine) synthesis and decreased degradation, increased neurotransmitter levels, improved ATPase function, and decreased phospholipase A2 activity and reactive oxygen species generation.¹²⁸ Preclinical studies of citicoline treatment showed lower infarct volumes and improved animal behavior, but a variety of different treatment protocols have been evaluated with less success.⁶⁸ Four clinical trials of citicoline have shown no differences in their primary outcome measures.^{19–21,153} Although a pooled analysis of 1372 participants with baseline NIHSS scores ≥ 8 showed a statistically significant effect of citicoline on 3-month global outcome incorporating NIHSS, mRS, and Barthel Index scores at 3 months (25.2% vs 20.2%; $p = 0.0034$), these trials were limited by multiple design flaws.²³ The most recent International Citicoline Trial on Acute Stroke (ICTuS), which evaluated 2298 patients in a multicenter RCT, showed that global recovery was similar in both groups (OR 1.03; $p = 0.364$).²² Finally, a recent meta-analysis of 10 randomized trials showed a higher rate of independence (OR 1.56, 95% CI 1.12–2.16), but the effect was more limited than those of other treatments (e.g., TPA).¹³⁰ Citicoline is in clinical use in a number of countries, including Argentina, Austria, Chile, Indonesia, Mexico, Portugal, Thailand, and Venezuela.¹⁸

Nitric Oxide

Lubeluzole, a 3,4-difluoro benzothiazole thought to downregulate glutamate-activated nitric oxide, was evaluated for its potential benefit in acute stroke. Preclinical models showed improved rodent tactile/proprioceptive behavior as well as reduced infarction volume when lubeluzole was administered within 5 minutes of stroke.²⁶ Two subsequent trials evaluated the use of lubeluzole but showed no difference in primary outcome of mortality at 3 months.^{31,56} The most recent trial in 1786 patients failed to show a difference in primary outcome of trichotomized Barthel Index at 3 months or in secondary outcomes.³¹ A meta-analysis of 5 trials and 3510 patients also failed to show a difference in mortality or dependence.⁴⁵

Immunoregulators for Stroke Therapy

Evaluation of enlimomab, an intercellular adhesion molecule-1 monoclonal antibody inhibitor that reduces leukocyte adhesion, showed some promise in preclinical

models¹⁶¹; however, in the Enlimomab Acute Stroke Trial (EAST) multicenter RCT of 625 patients, a difference in primary outcome of mRS score at 90 days showed a worsening of outcome in the treated group and a higher death rate.³⁹ A similar approach using neutrophil activation blockade with the CD11b/CD11 receptor inhibitor UK-279,276 was evaluated in 966 patients in the Acute Stroke Therapy by Inhibition of Neutrophils (ASTIN) trial⁷⁷; however, a lack of efficacy was seen and the trial was terminated early.

Hemodilution as Treatment for Stroke

Hemodilution was also evaluated as a treatment that could reduce excitotoxicity and metabolic demand during stroke. Several large trials, including the Scandinavian Stroke Study,¹²⁶ the Italian Acute Stroke Study,⁷² the Amsterdam Stroke Study,⁵⁵ the Italian Acute Stroke Study-Hemodilution (IASS-H) trial,⁹ and the Multicenter Austrian Hemodilution Trial,¹ all failed to show benefits in a variety of primary outcomes.

Rho Kinase Inhibitors

Rho kinases play important roles in cytoskeletal structure and motility, proliferation, and apoptosis via a variety of downstream molecules.^{78,84} Rho kinase inhibitors have been shown to increase endothelial nitric oxide and to provide neuroprotection in a variety of preclinical models.^{78,121} A trial of fasudil in 160 patients with acute ischemic stroke showed significant improvement in neurological function ($p = 0.0013$) and clinical outcome ($p = 0.0015$).¹³² Currently, fasudil is approved for the treatment of stroke outside of the US.

Challenges of Historical Approaches

The historical role of many of these agents has been extensively described.⁵¹ As is apparent, various limitations in study design and translation of preclinical findings to clinical trials have affected these trials.¹⁸ Time to treatment is an important and difficult feature to control, but must be considered when translating laboratory findings to human trials. Adequate preclinical data to support larger clinical trials have also been lacking in some cases, including objective outcomes evaluating improvement in infarct size and neurobehavioral function, in addition to other trial design features. Drug bioavailability, therapeutic dosing with regard to side-effect profiles, and blood-brain barrier penetration have all limited translation to clinical use.

Importantly, these prior failures have helped the development of current preclinical and clinical approaches. The Stroke Therapy Academic Industry Roundtable (STAIR) criteria were one such approach in improving the development of guidelines for preclinical trials to reduce the number of failed clinical trials.^{42,139} The STAIR criteria that must be assessed include drug doses, timing of treatment, type of animal model used, monitoring of animals during injury and recovery, objective outcomes, type of stroke model used, sex differences, genetic background, and combined pharmacological agents (e.g., TPA and neuroprotective treatments). Further guidelines from a stakeholders meeting of the US National Institute of Neuro-

logical Disorders and Stroke helped to identify additional areas for improvement of preclinical trials, including reproducibility and data reporting.⁸⁰

Recent and Future Clinical Trials

These novel approaches have built off of prior work in neuroprotection to investigate new strategies aimed at the underlying inflammatory nature of stroke and prevention of secondary injury (Table 3).

Hypothermia and Preservation of Cerebral Function Following Stroke

Hypothermia has been evaluated as a potential method for preserving cerebral function after ischemic stroke because it reduces the activity of a variety of molecular factors associated with ischemic stroke.¹⁴¹ A meta-analysis of 60 animal models of ischemic stroke described various degrees and timings of hypothermia³⁴ and different stroke induction models and lesion volumes. This study identified robust benefits of hypothermia in preclinical animal studies. Eleven of the studies analyzed in the meta-analysis with available data showed a combined treatment effect size of 50.7% (95% CI 37.9–63.5; $p < 0.001$); however, the meta-analysis acknowledged the disparity between the favorable preclinical trials and the generally negative results of clinical trials. A single-center, randomized clinical study of 25 patients demonstrated a better 6-month functional outcome on the NIHSS in patients treated with hypothermia and hemicraniectomy than in those with normothermia after hemicraniectomy.³⁷ Lower rates of hemorrhagic transformation and malignant cerebral edema have also been reported in another series of 75 patients.⁶²

Conversely, the Intravascular Cooling in the Treatment of Stroke (ICTuS) study was a Phase I trial evaluating the effect of inducing intravascular hypothermia within 6 hours of stroke onset.⁹⁷ No difference in mortality or 90-day outcome was seen for therapeutic hypothermia. The more recent ICTuS follow-up evaluated 120 subjects randomly assigned to hypothermia versus normothermia before the study was terminated.⁹⁶ Several large meta-analyses evaluating hypothermia during brain surgery⁴⁴ and cardiac surgery¹¹ have been published. Whereas results for improved neurological function after brain surgery were nonsignificant, mild therapeutic hypothermia was concluded to improve neurological outcome after cardiac arrest but not during in-hospital cardiac arrest, asystole, or noncardiac causes of arrest.^{11,46} The EuroHYP1 trial is currently enrolling 1500 subjects to assess the primary outcome of improved 3-month functional outcome for hypothermia versus normothermia.¹⁴⁸

Magnesium Treatment After Stroke

The recent Field Administration of Stroke Therapy–Magnesium (FAST-MAG) trial evaluated 1700 patients randomized to receive intravenous magnesium sulfate or placebo within 2 hours of stroke onset.¹²⁵ Patients received a loading dose at the time of suspected acute stroke followed by 24-hour maintenance. Seventy-three percent of patients had ischemic stroke, 2.8% had intracranial hemorrhage, and 3.9% had stroke-mimicking symptoms. No

TABLE 3. Current and future approaches in neuroprotection for ischemic stroke

Treatment	Mechanism	References
Excitotoxicity modulators		
Hypothermia	Various molecular mechanisms including reduction of excitotoxicity	Tahir & Pabaney 2016, ¹⁴¹ Dumitrascu et al. 2016, ³⁴ Els et al. 2006, ³⁷ Hong et al. 2014, ⁶² Lyden et al. 2005, ⁹⁷ Lyden et al. 2016, ⁹⁶ Galvin et al. 2015, ⁴⁴ Arrich et al. 2016, ¹¹ van der Worp et al. 2014 ¹⁴⁸
Magnesium	Regulates NMDA receptor by modulating voltage-dependent channel depolarization excitotoxicity	Saver et al. 2015 ¹²⁵
NA1	Inhibitor of postsynaptic density-94 protein that links NMDA receptors to excitotoxic signaling in neurons	Hill et al. 2012, ⁶¹ Liu et al. 2012 ⁹⁰
Uric acid	Antioxidant	Romanos et al. 2007, ¹²³ Onetti et al. 2015, ¹¹⁸ Chamorro et al. 2014, ¹⁷ Amaro et al. 2016, ⁴ Llull 2015 ⁹²
Fingolimod	Sphingosine-1-phosphate receptor target, reduces excitotoxicity or hypoxia	Wei et al. 2011, ¹⁵⁴ Fu et al. 2014 ⁴³
Peritoneal dialysis	Clearance of glutamate	del Carmen Godino et al. 2013 ²⁷
Anti-inflammatory agents		
IL-1 antagonist/anakinra	Anti-inflammatory agent	Mulcahy et al. 2003 ¹¹
Minocycline	Tetracycline antibiotic w/ anti-inflammatory effects	Fagan et al. 2010, ⁴⁰ Murata et al. 2008, ¹¹² Kohler et al. 2013, ⁷⁶ Amiri-Nikpour et al. 2015 ⁸
Anesthetic agents		
Selective serotonin-reuptake inhibitors		Siepmann et al. 2015 ¹³⁵
Repinotan hydrochloride	5-HT1A agonist	Teal et al. 2009 ¹⁴³
Simvastatin	HMG-CoA reductase inhibitor that reduces cholesterol levels	Montaner et al. 2016 ¹⁰⁸
Reperfusion-targeted agents		
Albumin	Volume expander, reduces volume of infarction, reduces cerebral edema, & improves behavioral outcomes in animals	Nimmagadda et al. 2008, ¹¹⁴ Park et al. 2008, ¹¹⁹ Ginsberg et al. 2006, ⁵³ Ginsberg et al. 2011, ⁵⁴ Ginsberg & Hill 2015 ⁵²
Erythropoietin	Secretory molecule that increases red blood cell production & is used in the treatment of anemia	Asadi et al. 2013, ¹³ Ehrenreich et al. 2009, ³⁶ Lund et al. 2014 ⁹⁵
Cerebral edema–targeting agent		
Glyburide	Inhibitor of SUR1-TRPM4 to reduce cerebral edema	Khanna et al. 2014, ⁷⁵ Tosun et al. 2013, ¹⁴⁵ Sheth et al. 2014, ¹³¹ Sheth et al. 2016 ¹³⁰
Nitric oxide mediator		
Glyceryl trinitrate/nitroglycerine	Nitric oxide donor	Bath et al. 2015 ¹⁴
Immunomodulator		
Natalizumab	CD49d antibody that targets $\alpha 4$ -integrin & reduces leukocyte invasion	Liesz et al. 2011, ⁸⁵ Llovera et al. 2015 ⁹¹

HMG-CoA = 3-hydroxy-3-methylglutaryl coenzyme A.

significant shift in 90-day mRS score was seen after treatment with magnesium ($p = 0.28$). Secondary outcomes of mortality also did not differ between the magnesium and placebo groups (15.4% vs 15.5%; $p = 0.95$).

Albumin as a Neuroprotective Agent

Several key trials have evaluated the potential for albumin to serve as a neuroprotective strategy in stroke. Preclinical models suggest that 25% albumin shows neuroprotective potential by decreasing the volume of infarction, reducing cerebral edema, and improving behavioral outcomes.^{113,118} The Phase I Albumin in Acute Stroke (ALIAS) trial demonstrated that 25% albumin within 16 hours of stroke onset was safe for use in 82 patients.⁵³ A

later Phase III ALIAS Part 1 trial in 434 patients who received 25% albumin within 5 hours of stroke demonstrated similar safety rates between groups but showed increased risk in patients > 83 years of age, which has led to a restructuring of future trials of albumin.⁵⁴ Recently published results of the Part 2 safety trial in 830 patients showed that endovascular thrombolysis, but not TPA, increased the rate of intracranial hemorrhage (risk ratio 2.14; $p = 0.025$).⁵²

Glyburide to Preserve Cerebral Function After Injury

Since implication of the SUR1-TRPM4 channel in cerebral edema and hemorrhagic conversion of stroke, cerebral edema after ischemic stroke has been targeted with

glyburide to preserve cerebral function after injury.^{75,145} A pilot trial in 10 patients helped demonstrate that a dose of 3 mg per day of intravenous glyburide was well tolerated.¹³¹ The GAMES-RP (glyburide on brain swelling after large hemispheric infarction) Phase II trial evaluated 86 patients with a primary outcome of mRS score 0–4 at 90 days in patients who did not undergo a decompressive craniectomy.¹³⁰ No difference in primary composite outcome was seen, and the trial was terminated early because of loss of funding.

Uric Acid

Uric acid, a potent antioxidant that contains two-thirds of the plasma's total antioxidant capacity, was evaluated in several preclinical models.^{117,123} Combined uric acid and alteplase within 4.5 hours of stroke was evaluated in the Phase IIb/III URICO-ICTUS (Uric acid in patients with acute stroke) trial.¹⁷ A subsequent analysis of the URICO-ICTUS trial for early ischemic worsening, defined as an increase of ≥ 4 points on the NIHSS within 72 hours, showed a significant decrease in early ischemic worsening in patients treated with uric acid (7 of 204 vs 18 of 200; $p = 0.01$).⁴ Although the primary outcome of mRS score at 90 days was no different from that of control subjects, the incidence of early clinical worsening was lower and a greater number of patients with treatment reached full independence. Reduced infarct size was shown in specific subgroups, including women, patients with pretreatment hyperglycemia, and early recanalization.^{5,92}

Other Approaches

The ENACT (Safety and efficacy of NA-1 in patients with iatrogenic stroke after endovascular aneurysm repair) trial evaluated intravenous NA-1, an inhibitor of postsynaptic density-94 protein that links NMDA receptors to excitotoxic signaling in neurons. Although preclinical trials showed benefit for NA-1, clinical trials failed to show reduction in infarction size.⁶¹

Calcium channel antagonism with ginsenoside Rd has also been evaluated in an RCT during treatment within 72 hours of stroke, with improved functional recovery and without significant adverse events.⁹⁰

The Efficacy of Nitric Oxide in Stroke (ENOS) trial evaluated glyceryl trinitrate or nitroglycerin, a nitric oxide donor, in the treatment of acute ischemic stroke.¹⁴ The ENOS trial did not show a difference in primary outcome. Preclinical trials showed that glyceryl trinitrate could reduce lesion size and increase cerebral perfusion.⁸⁸ A subgroup analysis of 25 studies using a variety of nitric oxide donors in 273 animals treated within 6 hours of stroke showed improved functional outcome, reduced infarct volume, improved cerebral blood flow, and reduced mortality.¹⁵⁶

Fingolimod, which targets the sphingosine-1-phosphate receptor and reduces lymphocyte concentration and excitotoxicity or hypoxia, has been evaluated in preclinical and clinical trials.¹⁵⁴ An open-label trial of fingolimod administered within 72 hours of stroke showed efficacy in limiting infarct size and improving disability.⁴³

Peritoneal dialysis to reduce blood levels of glutamate has shown efficacy in a rodent model.²⁷ Current trials

are evaluating dialysis in acute stroke (EudraCT 2012–000791–42).

Natalizumab is a CD49d antibody that targets $\alpha 4$ -integrin and reduces leukocyte invasion. It has been shown in preclinical stroke models to reduce T-cell and neutrophil infiltration.⁸⁵ In preclinical models, natalizumab also showed reduction in infarct size after stroke.⁹¹ The ACTION trial (NCT01955707) is currently evaluating this treatment in clinical settings, and preliminary results suggest that treatment did not reduce infarct volume at 5 days but improved the Stroke Clinical Impact-16 score and cognitive function at 90 days.

IL-1 antagonism, via recombinant IL-1Ra/anakinra, is another recently defined anti-inflammatory mechanism that is being evaluated in ischemic stroke. Animal models of IL-1 have shown reduced lesion size and cell death.¹¹¹ A current Phase II trial is evaluating this treatment in clinical settings (ISRCTN74236229).

Minocycline, a tetracycline antibiotic with anti-inflammatory effects, showed good preclinical utility and was beneficial in a small pilot study, Minocycline to Improve Neurological Outcome in Stroke (MINOS). The results showed that minocycline was safe for use within 24 hours of stroke but demonstrated no improvement in disability at 90 days.⁴⁰ Animal models have also shown improved cerebral protection during combined TPA and minocycline administration.¹¹² Whereas an underpowered open-label trial in 95 participants failed to show a difference in mRS score of ≤ 2 at 90 days with treatment,⁷⁶ another open-label trial evaluating minocycline (200 mg for 5 days) in 53 patients showed significantly better NIHSS scores at 90 days compared with control subjects (4 vs 7; $p = 0.031$).⁸ A larger RCT of minocycline with TPA is pending (ACTRN12611001053910).

Erythropoietin, a secretory molecule that increases red blood cell production and is used in the treatment of anemia, has been evaluated for use in treating ischemic stroke. A Phase II trial comparing 37 patients treated with erythropoietin and 43 patients who received placebo demonstrated a significant decrease in NIHSS score at 4 weeks for patients who were treated with erythropoietin (2.11 vs 6.05; $p = 0.0001$).¹⁴ A Phase III trial of 522 patients with middle cerebral artery stroke showed no favorable effect of erythropoietin on the primary outcome of Barthel Index at Day 90 ($p = 0.45$).³⁶ In addition, no difference in death rate (42 of 256 vs 24 of 266, OR 1.98, 95% CI 1.16–3.38) was seen between the erythropoietin and placebo groups. A meta-analysis of high-dose erythropoietin for tissue protection that evaluated 26 RCTs and 3176 patients failed to show a tissue-protective role.⁹⁵

Anesthetic agents have also been assessed for potential efficacy in preclinical and clinical models of tissue protection.⁷⁰ A meta-analysis of selective serotonin-reuptake inhibitors involving 52 trials of 4060 patients suggested improvement of structure-function, neurogenesis, migration of cells into damaged areas, modulation of the neurohormonal system, and improved cerebral blood flow.¹²⁵ The modified Randomized Exposure Controlled Trial (mRECT) evaluated 681 patients treated with TPA plus repinotan hydrochloride (a 5-HT_{1A} serotonin agonist) or placebo.¹⁴³ The primary outcome of response rate on

Barthel Index was similar between the treated and control groups (127 of 342 vs 143 of 337; $p = 0.149$). High-dose simvastatin (40 mg) in combination with thrombolysis was assessed in the Stroke Treatment With Acute Reperfusion and Simvastatin (STARS) trial;¹⁰⁸ there was no difference in the primary outcome of improved mRS score ≥ 2 in a total of 104 patients randomly assigned to receive either simvastatin or placebo.

Summary of Current and Future Clinical Approaches

Overall, these approaches for neuroprotection in acute stroke aim to use known pathophysiological mechanisms of disease, such as excitatory inflammation, as well as novel approaches, such as reduction of SUR1-TRPM4-mediated cerebral edema. As of December 2016, the Internet Stroke Center listed 346 studies evaluating treatments in ischemic stroke, including 60 trials of neuroprotective agents. The publication of the results of these trials, whether findings are confirmatory or negative, will be important for the understanding of ischemic stroke and future therapeutic approaches.

Integration With Endovascular Therapies

Recent clinical trials supporting the use of endovascular treatment for acute ischemic stroke have redefined the potential applications of neuroprotective strategies. Key trials, including Multicenter Randomized Clinical Trial of Endovascular Treatment for Acute Ischemic Stroke in the Netherlands (MR CLEAN), Endovascular Treatment for Small Core and Proximal Occlusion Ischemic Stroke (ESCAPE), Extending the Time for Thrombolysis in Emergency Neurological Deficits–Intra-Arterial (EXTEND-IA), Solitaire With the Intention For Thrombectomy as Primary Endovascular Treatment (SWIFT-PRIME), and Endovascular Revascularization With Solitaire Device Versus Best Medical Therapy in Anterior Circulation Stroke Within 8 Hours (REVASCAT), have improved our understanding of the benefits of early endovascular intervention in ischemic stroke.^{49,122}

Protection of the ischemic penumbra (areas of tissue with neurological dysfunction due to reduced blood supply but maintained metabolism and potential for recovery) is the critical approach in endovascular treatments.⁶⁶ Phases of infarct progression include the ischemic core (20% less than normal flow), the ischemic penumbra (25%–50% of normal flow), and less ischemic/oligemic tissue with variable apoptosis.⁶⁰ It is the penumbra and oligemic areas that could potentially benefit from neuroprotective strategies by improving collateral flow and protecting viable tissue.

To work synergistically with current reperfusion strategies, approaches would need to: 1) involve early intervention (within the first 4–6 hours of stroke), 2) show preclinical evidence of efficacy and adequate therapeutic levels after administration with manageable side effects, and 3) have adequate trials to evaluate use.⁴⁹ Current endovascular therapies have shown improved recanalization rates compared with first-generation methods alone. In addition, improved time to treatment and the formation of dedicated stroke centers have led to a potential resurgence of neuroprotective studies. Various experimental therapies may

be useful in early revascularization. Oxidative stress–reducing agents or treatments that improve cerebral perfusion are potential approaches. These treatments may have failed initial clinical trials if adequate tissue perfusion and drug delivery were hindered by the initial ischemic stroke. In addition, direct catheter delivery of these agents may be a method of rapid and targeted drug delivery.

Conclusions

Although numerous and varied clinical approaches have been evaluated for use in neuroprotection with ischemic stroke, few treatments have entered clinical practice. As additional guidelines for the translation of preclinical to clinical treatment have been defined, improved collaboration and study networks have arisen as a result of previously examined treatment approaches. Assessment of future treatments by collaborations among multiple institutions and partnerships with patient-advocacy groups may allow more rapid identification of potentially beneficial agents.

The use of combined therapeutic options, such as the combination of TPA with other neuroprotective treatments, should continue to be an area of exploration. In fact, treatment to help reduce hemorrhagic conversion of ischemic stroke could potentially make TPA a safer therapy. Newer findings showing the benefit of thrombolytic reperfusion strategies have also created new opportunities for combined treatments. Because of the rapid rise in the number of stroke centers, telestroke sites, and even mobile telestroke services, additional pharmacological treatments can be rapidly delivered to patients and may change the paradigm for treatment. Thus, although past trials of neuroprotective agents have been limited, the future is promising.

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Disclosures

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author Contributions

Conception and design: Park, Karsy. Acquisition of data: Karsy, Brock, Guan. Drafting the article: Karsy, Brock, Guan. Critically revising the article: Park, Taussky, Kalani. Reviewed submitted version of manuscript: Park, Karsy, Taussky, Kalani. Approved the final version of the manuscript on behalf of all authors: Park.

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