Review: Clinical Trial Outcomes

CLINICAL INVESTIGATION

Neuroprotective agents in ischemic stroke: past failures and future opportunities

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Tissue plasminogen activator is the only US FDA-approved therapy for acute ischemic stroke treatment. There is a need for other therapies such as neuroprotective agents that can be used in acute ischemic stroke patients. Many neuroprotective agents have been shown to be promising after testing in animal models of acute stroke, but when tested in patients, efficacy signals are not consistent or safety challenges have been noted. This review article will discuss different neuroprotective agents, including free-radical scavengers and antioxidants, NMDA-receptor antagonists, inflammatory cascade inhibitors and different ion channel blockers/modulators that have been tested in acute ischemic stroke. The review will also address the key reasons for their failure in clinical trials and provide recommendations to improve translation of basic science research into day-to-day clinical practice.

Keywords: antioxidant • free-radical scavenger • inflammatory cascade inhibitor • ion channel blocker/modulator • neuroprotection • NMDA-receptor antagonists • stroke

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Stroke is the largest single cause of adult disability and the second most common cause of death in developed countries. A major breakthrough in the medical treatment of ischemic stroke was intravenous (iv.) thrombolysis with tissue plasminogen activator (t-PA), which is the only US FDA-approved drug therapy for acute ischemic stroke patients. It must be administered within a 4.5-h window from symptom onset [1,2]. Primarily due to this short window of time for acute stroke treatment, only a minority (~5%) of patients with acute ischemic stroke are eligible for thrombolysis with t-PA [3,4]. Modern endovascular therapy with mechanical thrombectomy and intra-arterial fibrinolysis are other acute treatment options, however, recent trials have raised controversy about the value of these therapies [5–7]. Therefore, the primary standard of care in acute ischemic stroke remains iv. t-PA if the stroke patient presents to the hospital within the aforementioned time period. There is a substantial need for additional effective and safe treatments that will benefit a large number of acute stroke patients.

The concept of neuroprotective agents has been a focus of attention over past decades, with many experimental neuroprotective compounds being tested both preclinically and in humans. The standard of care – iv. t-PA – may provide partial and sometimes complete recanalization of major cerebral arteries to restore reperfusion in acute ischemic brain injury by salvaging potentially viable neurons in the penumbra that surrounds the infarct core. Over 1000 neuroprotective agents have been studied in preclinical stroke research, many with promising results [8]. However, translation of these neuroprotective drugs has failed in the clinical setting.

Nearly 200 neuroprotection clinical trials have been completed or are ongoing [9,201], however, few have achieved success.

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Why have neuroprotective agents failed in human stroke trials?

There are different possible reasons that have led to intense discussion over the last several years. It is very difficult to create a true representative model for human ischemic stroke. Most stroke patients have different or other medical disorders and stroke risk factors. Many have multifocal atherosclerosis developing over decades that is not present in healthy animals used in laboratory research in different stroke models. Moreover, many stroke patients take medications to control risk factors and other medical conditions that can affect the ischemic process and interact with therapeutic neuroprotective agents. There are also differences in techniques and end points used in different animal models and clinical trials. These include, but are not limited to, different anesthetic agents and clinical end points that are meaningful for patients. The other important points are missing the appropriate early window of time for efficacy and salvage of brain tissue, inappropriate patient selection, lack of penetration of study drug into target tissues and lack of establishment of reperfusion. Moreover, studying synergistic combinations of these drugs and collaboration between academics and industry are important in designing study protocols [10–13]. However, the window of opportunity may be reopening as several recent clinical trials show safety and efficacy promise with these agents [14,15].

We now review our current understanding of neuroprotective agents in animal models of acute stroke and discuss the status of the neuroprotective field from both a preclinical and clinical standpoint (Tables 1 & 2). In addition, we identify challenges associated with translation of neuroprotective agents from bench to the bedside and provide recommendations in regards to current criteria for continuation of preclinical neuroprotection studies.

Free-radical scavengers & oxidative stress

During stroke, release of the excitatory neurotransmitter glutamate is increased and its reuptake is reduced [16]. Accumulation of excitotoxic neurotransmitters results in excessive stimulation of glutamate receptors (primarily the NMDA receptor). Furthermore, matrix metalloproteinases (MMPs) activity in the brain after ischemic injury enhances tissue damage through multiple different mechanisms [17]. The neuroprotective effect in ischemic stroke has been suggested to be associated with multiple different pathways involving the cerebral ischemic process (e.g., cellular excitotoxicity, oxidative stress, inflammatory cascade, MMP activation and blood–brain barrier damage). Our previous study in a murine model of stroke revealed that carnosine, a naturally occurring dipeptide with several neuroprotective properties, significantly decreased infarct size and ameliorated neuronal damage when administrated both before and after induction of ischemia [18,19]. It has been further demonstrated that this neuroprotective effect in a murine model of ischemic stroke, at least in part, is related to the decreased level of reactive oxygen species, preserved glutathione levels, and attenuated MMP levels as well as its activity [18]. These beneficial effects were maintained for 7 days postischemic stroke in mice [19].

It has been shown that ischemia induced mitochondrial injury and the subsequent activiation of proapoptotic proteins in mitochondria like cytochrome c and apoptosis-inducing factor, may enhance neuronal apoptotic death after ischemic stroke [16,20]. Anthocyanins (isolated and purified from tart cherry), including cyanidin-3-*O*-glycosides, are strong antioxidants and also possess anti-inflammatory properties [21]. Our murine ischemic stroke model has shown that infarction volume was significantly attenuated by 27% in a mouse stroke model pretreated with anthocyanin when compared with a vehicle-treated group. Furthermore, delayed treatment with anthocyanin showed a 25% reduction in infarct volume. Neurological functional outcome was significantly improved in mice with stroke who were pre- or post-treated with anthocyanin. These benefits are believed to be mediated by a decrease in brain levels of superoxide as well as blockage of apoptosis-inducing factor release in mitochondria [22].

More recently, a preclinical study in rats of both permanent and transient ischemia showed that carnosine treatment exhibited significant cerebroprotection against histological and functional damage, with a wide therapeutic and clinically relevant time window of 6 and 9 h after ischemic stroke. Future clinical trials are warranted with these agents, such as carnosine for further development of acute stroke therapy [23].

However, there has been translational failure the transition of neuroprotective agents from animal models to clinical patients with stroke. Dextrorphan, a noncompetitive NMDA antagonist and a metabolite of the cough suppressant dextromethorphan, was the first NMDA antagonist studied in human stroke patients [24]. Unfortunately, intolerable adverse effects associated with dextrorphan including hallucinations, agitation and hypotension limited its use. A trial of a glycine antagonist, GV150526, in 1367 patients with acute stroke was completed in 2000. It was safe and well tolerated, but no clinical improvement was observed after 3 months of follow up [25].

The effect of ebselen, a seleno-organic compound with antioxidant activity mediated by a glutathione peroxidase-like action [26] on the outcome of acute ischemic stroke was evaluated in a multicenter, placebo-controlled, double-blind clinical trial [27]. Early treatment with ebselen

in 302 patients with acute ischemic stroke showed that it improved the modified Mathew scale and modified Barthel Index scores at 1 and 3 months after treatment [27]. A clinical Phase III trial of ebselen in acute stroke is currently ongoing [201]. Edaravone, another free-radical scavenger, inhibits vascular endothelial cell injury and ameliorates neuronal damage in ischemic animal models [28]. A multicenter, randomized, placebo-controlled, double-blind study in 250 acute ischemic stroke patients (125 in edaravone-treated and 125 in placebo-treated group) commencing within 72 h of symptoms onset was completed in 2003 [29]. A significant improvement in functional outcome according to the modified Rankin scale was observed in the edaravone treatment group. Edaravone is a neuroprotective agent that is potentially useful for treating acute ischemic stroke, since it can have significant beneficial effects on functional outcomes compared with placebo. Nakase and colleagues further demonstrated that edaravone treatment (83 edaravone-treated compared with 83 nontreated stroke patients) reduced the volume of the infarct and improved neurological deficits during the subacute period, especially in small-vessel occlusion strokes, at 1 year following stroke onset [30]. Currently, a Phase III clinical trial of edaravone in acute ischemic stroke is being undertaken [201].

NXY-059 is a free-radical-trapping agent that reduced cerebral infarction size and preserved neurological function in animal models of acute ischemic stroke [31].

Table 1. Neuroprotection studies: animal models of ischemic stroke.

Agent	Model	Proposed mechanisms	Results/outcomes	Ref.
Carnosine	Mouse, permanent MCA occlusion	Antioxidant and decrease of MMP level	Decreased infarct size and improved neurological function	[18,19]
Anthocyanin	Mouse, permanent MCA occlusion	Decreased brain superoxide level and blockage of mitochondria AIF release	Decreased infarct size and improved neurological function	[22]
Edaravone	Mouse, transient MCA ischemia	Reduced oxidative products with anti-inflammatory responses	Reduced infarct size and improved neurological function	[28]
NXY-059	Monkey, permanent MCA occlusion	Free radical-trapping properties	Ameliorated infarct and lessened disability	[31]
Cerebrolysin	Rat, permanent MCA occlusion	Increased neurogenesis	Did not reduce infarct size, but improved neurological function	[38]
Magnesium	Rat, transient MCA ischemia	Antagonist of calcium channel noncompetitive NMDA receptors and inhibits excitatory neurotransmitter	Decreased infarct volume	[40]
Magnesium	Goat, isolated MCA *in vitro* and measuring cerebral blood flow *in vivo*	Meditates endothelin-1 and 5-hydroxytryptamine pathways	Decreased cerebral vascular constriction and increased cerebral blood flow	[44]
Albumin	Rat, transient MCA occlusion	Hemodilution, antioxidant effects and metabolic benefits	Reduced infarct size and improved neurological function	[49,52]
Antiadhension antibody	Rat, transient MCA ischemia	Antiadhesion and subsequently inhibits inflammatory cascade	Decreased infarct size and improved cerebral blood flow	[59]
Tacrolimus (FK506)	Rat, transient MCA ischemia	Anti-inflammatory cascade	Decreased infarct volume when given within 4 h after ischemia	[62,63]
Minocycline	Rat, transient MCA ischemia	Anti-inflammatory effects	Decreased infarct size	[68]
Riluzole	Global ischemia in Mongolian gerbil and focal MCA ischemia in rat	Inhibition of sodium channel activation	Reduced infarct size	[83]
Sipatrigine (BW619C89)	Rat, permanent MCA occlusion	Sodium channel antagonist	Reduced infarct volume and improves neurological function	[84]
BMS-204352	Rat, permanent MCA occlusion	Calcium sensitive openers of maxi-K channels	Reduced infarct volume, but had no effects on blood pressure or cerebral blood flow	[92]

AIF: Apoptosis inducing factor; MCA: Middle cerebral artery; MMP: Matrix metalloproteinases.

Table 2. Clinical trials of neuroprotection in patients with acute ischemic stroke.

Agent	Observed duration for efficacy outcomes	Proposed mechanisms	Results/outcomes	Ref.
Dextrorphan	3 months	Noncompetitive NMDA antagonist	Safe and tolerated, but no improvement was observed	[24]
Ebselen	1 and 3 months	Antioxidant	Improved modified Mathew scale and Barthel index A Phase III trial is under investigation	[26,27,201]
Edaravone	3 months 1 year	Free-radical scavenger	Reduced infarct volume and improved neurological function A Phase III trial is ongoing	[29–31]
NXY-059	90 days	Free radical-trapping properties	Safe, but ineffective	[32–34]
Citicoline	90 days	Accelerate resynthesis of phospholipids, suppress the release of free fatty acids and reduce free radical generation	Not efficacious in the treatment of moderate-to-severe acute ischemic stroke	[36]
Cerebrolysin	90 days	Prevent free radical formation and counteract excitotoxicity	Neutral results overall, but favorable outcome trend was observed in the severely affected stroke patients	[39]
Magnesium	3 months	Antagonist of calcium channel noncompetitive NMDA receptors, and inhibits excitatory neurotransmitter	Good functional outcome (modified Rankin score), and no serious adverse events A Phase III trial is in progress	[48]
Albumin	3 months	Multifactorial including hemodilution, antioxidant effects and metabolic benefits	Favorable primary outcome, safe and well tolerated A Phase III trial was terminated by DSMB after an interim analysis	[52,53,201]
Enlimomab	3 months	Anti-inflammatory cascade	Worse stroke outcome due primarily to infections and fever	[61]
Lovastatin	3 days	Modulates immune system, increases cerebral perfusion and survival signals	High doses of lovastatin are safe and feasible up to 3 days after stroke in a Phase IB trial	[66,201]
Minocycline	3 months	Anti-inflammatory effects	Safe and well tolerated, improvement of neurological function A Phase III trial is in progress	[71,72,201]
Sipatrigine (619C89)	Terminated early	Sodium channel antagonist	Toxicity and no clinical efficacy observed	[85]
Nimodipine	3 months	Calcium channel blocker	No clinical benefits were observed	[87]
BMS-204352	12 weeks	Calcium sensitive openers of maxi-K channels	Failed to show superior efficacy	[93]
DP-b99	3 months	Zinc chelator	Failed to show efficacy	[94]
LeukArrest	Terminated at 28 days	Anti-inflammatory cascade	Terminated early due to unfavorable interim results	[201]

DSMB: Data safety and monitoring board.

The SAINT I study demonstrated that NXY-059 administrated within 6 h after the onset of ischemic stroke significantly improved the primary outcome, a reduction in disability as measured by the modified Rankin score at 90 days, but not the neurological outcome measured by the NIH stroke score [32]. However, the SAINT II study, a large clinical trial that enrolled 3306 patients, failed to validate the findings that resulted from SAINT I. The SAINT II trial concluded that NXY-059 is safe but ineffective for treating patients with acute ischemic stroke [33]. Furthermore, Diener and colleagues showed that NXY-059 was ineffective for treatment of acute ischemic stroke within 6 h of symptom onset (2438 stroke patients received NXY-059 treatment compared to 2450 patients who received placebo) [34].

Citicoline is an intermediate in the biosynthesis of phosphatidylcholine (PtdCho), which has been shown to reduce infarct volume in animal models of acute ischemic stroke [35]. The mechanism by which citicoline is thought to act includes enhancement of synthesis of PtdCho and sphingomyelin, attenuation of lipid peroxidation and restoration of Na^+/KCl^+ ATPase activity [35]. ICTUS was developed in 2006 [202]. The study enrolled 2298 patients from November 2006 to October 2011 (1148 patients received citicoline and 1150 patients were in the placebo group). Citicoline was not efficacious in the treatment of moderate-to-severe acute ischemic stroke [36].

Animal studies have demonstrated that cerebrolysin, a porcine brain-derived preparation of low-molecular-weight neuropeptide and free amino acids, could improve neurological function and reduce infarct size by potentially preventing free radical formation and by counteracting excitotoxicity that can also prevent cell death [37,38]. Recent published data from a trial of cerebrolysin in patients with acute ischemic stroke in Asia showed neutral results between the cerebrolysin treated compared with the placebo group [39]. A favorable outcome trend was noted in the more severely affected patients with ischemic stroke who received cerebrolysin treatment [39]. A further large clinical trial should be warranted to investigate the efficacy of cerebrolysin in patients with acute cerebral ischemia.

Excitotoxicity & magnesium

The neuroprotective effect of intra-arterial magnesium sulfate has been shown in animal models of reversible focal cerebral ischemia [40]. Magnesium produces neuroprotection by a number of mechanisms including antagonism of calcium channels, noncompetitive antagonism of NMDA receptors, inhibition of excitatory neurotransmitter release, and vascular smooth muscle relaxation [41].

Magnesium also has some potentially pertinent vascular effects, including antagonism of vasoconstrictive mediators (i.e., endothelin-1) [42–44], and enhanced cerebral blood flow, which is presumably as a consequence of vasodilatation of cerebral blood vessels [45,46]. The purported benefit of magnesium has been augmented by previous results that have shown as many as 80% of stroke patients demonstrated significantly decreased serum ionized magnesium levels, with 15% showing an elevation in their ionized calcium/magnesium ratio, which is known to be a state that promotes vascular tone [47]. The advantages of using magnesium include its easy availability and low cost, and that it is also well tolerated.

The FAST-MAG pilot trial was designed to overcome the drawback of the delayed administration of neuroprotective agents in prior clinical trials, which has demonstrated that initiation of magnesium by paramedics in the field within 2 h of symptom onset is feasible and safe [48]. Based on the positive pilot results, a large Phase III clinical trial is in progress [201], designed to investigate if magnesium is effective when administrated by emergency medical service personnel between 15 min and 2 h after stroke symptom onset.

Albumin

The neuroprotective properties of human albumin have been shown in animal models of ischemic stroke [49]. Animal studies suggested that its neuroprotective effects are related to its antioxidant properties, preservation of microvascular integrity, decreasing endothelial cell apoptosis [50], hemodiluation and mobilization of free fatty acids that are required for restoration of impaired neurons [51]. The ALIAS pilot trial designed to evaluate the safety of escalating doses of albumin in patients with acute stroke was reported in 2006 [52]. In total 82 subjects received albumin, and 42 of them also received standard dose iv. t-PA. The study showed that albumin-related adverse effects (elevated brain natriuretic peptide-BNP and pulmonary edema) were mild or moderate in severity.

Additionally, the t-PA-treated stroke patients who received higher dose albumin were three-times more likely to achieve a good outcome than subjects receiving lower dose albumin. The latter findings suggest a positive synergistic effect between albumin and iv. t-PA. These promising preliminary results led to ALIAS-part 2, a Phase III trial in which albumin therapy was compared with placebo (isovolumic normal saline) in patients with acute ischemic stroke [53]. However, recruitment was halted by data safety monitoring board following interim analysis, and the trial was terminated.

Inflammatory cascade inhibition

Ischemic stroke is often triggered by several processes involving endothelial activation and proinflammatory and prothrombotic interactions between vessel wall and circulating blood elements, and ultimately thrombogenesis [54].

Within minutes after ischemic stroke, several pro-inflammatory cascades are initiated including upregulation of cell adhesion molecules [55], over expression of P-selectin [56] and intracellular adhesion molecules (ICAM-1) [57].

Elevated ICAM-1 expression has also been observed on microvessels within infarcts in patients surviving for 15 h to 6 days after ischemic stroke [58]. Animal studies have demonstrated that antiadhesion antibody decreases infarct size up to 70% after 2 h of transient focal ischemia and improves cerebral blood flow [59].

Enlimomab is a murine monoclonal anti-ICAM antibody that has undergone Phase III trial testing in patients with acute stroke [60]. The trial showed enlimomab treatment was associated with worse outcomes including greater mortality rates compared with the placebo-treated group.

The stroke patients who received enlimomab treatment in this study developed anti-mouse antibodies. Thus, the negative outcome from the trial might be related to upregulation of endogenous adhension molecules due to the use of anti-ICAM antibody originally derived from mice, which subsequently resulted in a paradoxical inflammatory response. Developing humanized antiadhension molecules would be a rational approach to avoid this adverse response [60,61]. As such, a humanized IgG1 antibody against human CD18 (Hu23F2G or LeukArrest) was developed to block leukocyte infiltration while avoiding the adverse effects from enlimomab. A Phase III trial of LeukArrest enrolled patients with ischemic stroke within 12 h of symptom onset allowed concomitant use of t-PA. The trial was terminated early after the interim results were unfavorable [201]. Tacrolimus (FK506) has been used for prevention of transplant organ rejection and has been studied as a potential neuroprotective agent due to its immunosuppressive properties. It has been shown to exert a potent neuroprotective activity when administered within 4 h after occlusion of the middle cerebral artery in a nonhuman primate model of stroke [62,63].

3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors or statins are becoming increasingly recognized as important in brain ischemia. Not only do statin agents act on lipid metabolism, decreasing LDL cholesterol levels, statins also have pleiotropic effects including, but not limited to, modulating the immune system [64], increasing cerebral perfusion via upregulating angiogenesis and by activation of survival signals [65]. A Phase IB dose-escalation trial using Neu-START was completed and showed that different doses (maximum 8 mg/kg) of lovastatin administration was safe up to 3 days after acute ischemic stroke [66].

Minocycline, a semisynthetic second-generation drug of the tetracycline class, exerts anti-inflammatory effects such as inhibition of microglial activation [67] and production of other inflammatory mediators [68]. Furthermore, it has been demonstrated that minocycline inhibits caspase, inducible NOS (iNOS), and P38 MARK [69], and has neuroprotective properties like hypothermia [70]. It appears that minocycline is an ideal neuroprotective candidate on the basis of its established safety profile in animal studies, CNS penetration and low cost. In an open-label clinical study, minocycline administration led to a significantly better outcome in acute stroke patients compared with the placebo-treated group [71]. Additionally, minocycline was shown to be safe and well tolerated alone or in combination with iv. t-PA in patients with acute stroke [72]. These positive and encouraging results have led to the ongoing Phase III Neu-MAST [201].

Ion channel blockers/modulators & chelators

It has been demonstrated that more than 50% of the total ATP synthesized in the brain is applied to maintain the energy-dependent ionic pumps that are responsible for maintaining neuronal intracellular and extracellular ionic balance under normal conditions [73]. Moreover, different studies have found that total ATP levels in the brain decreased significantly within 2 min after ischemia [73,74]. The reduction of ATP levels in the setting of cerebral ischemia impairs neuronal function of ionic homeostasis, which results in decreasing K^+ and increasing Na^+ concentrations that leads to the depolarization of neuronal membranes [75]. This anoxic depolarization is manifested by neuronal death within the ischemic region along with the opening of voltage gated Ca^{2+} channels to cause neuronal Ca^{2+} overload [75–77]. Animal studies further revealed that peri-infarct depolarization in the ischemic penumbra could result in rapid de- and re-polarizations of these jeopardized ischemic neurons, which would cause further neuronal damage [78]. Thereafter, it is reasonable to believe that ionic imbalance after ischemic insult plays a major role during the ischemic pathophysiological process.

Downregulation of the Na^+ channels is a possible effective way of not only reducing energy demand due to restoring the ionic gradient across the cellular membrane, but also to reduce the Na^+ influx into the neurons with resulting energy preservation. Additionally, it would prevent the intrinsic neurotoxicity of the acute Na^+ influx and the linked Ca^{2+} influx [79,80]. Experimental studies with phenytoin, carbamazepine, lamotrigine, sipatrigine (619C89) and riluzole showed benefits in a variety of animal models with ischemic stroke [81–84]. However, clinical trials with sipatrigine and fosphenytoin were terminated without success due to toxicity and lack of clinical efficacy [85].

Calcium channel blockers were extensively evaluated in acute stroke with the hope that stemming excessive cellular Ca^{2+} influx after brain ischemia might ameliorate neuronal injury. T-type Ca^{2+}channel blockers can protect ischemic neurons *in vitro* [86]. Studies of Ca^{2+} blockers,

however, did not show efficacy in the clinical setting. Horn and colleagues tested nimodipine in patients with acute stroke within 6 h of symptom onset. The study was discontinued early after analysis of 439 acute stroke patients as there were no beneficial effects of nimodipine [87]. A meta-analysis reviewing a total of 22 Ca^{2+} antagonist trials in patients with acute ischemic stroke revealed that no benefit was found by using Ca^{2+} antagonist including earlier treatment within 24 h after stroke symptoms onset [88]. A possible explanation for lack of efficacy could be relating to hypotension resulted from blocking the L-type Ca^{2+} channels in the setting of vasodilatation in patients with acute stroke, which could further impair autoregulation of the cerebral circulation within the ischemic region to cause exacerbation of hypoperfusion within the ischemic penumbra [89]. Other subtypes of Ca^{2+} channel antagonist (e.g., N-type Ca^{2+} antagonist) might be an alternative target for future studies.

K^+ channels, transmembrance proteins, execute an important role in the electrical activity of all excitable tissues including brain tissue. Pharmacological modulation of specific K^+ channels is recognized as a major strategy in the treatment of a broad range of disorders including ischemic stroke [90,91]. BMS-204352 (MaxiPost) has proven effective in reducing infarct volume in a rat model of stroke [92]. Although no clinically significant differences in organ toxicity or adverse effects were found, a fluorooxindole K^+ channel opener (BMS-204352) failed to show superior efficacy in acute stroke patients compared with placebo in POST, aPhase III study that included 1978 patients at 200 centers worldwide [93].

Zinc has a neurotoxic role during cerebral ischemia when there is a loss of ion hemeostasis. DP-b99 is a zinc chelator and was considered to be neuroprotective based on neurorestorative effects in preclnical and Phase II studies [94]. The safety and efficacy of DP-b99 were evaluated in a randomized, placebo-controlled, double-blind trial in which patients with moderate stroke severity were treated within 9 h of symptom onset of acute ischemic stroke involving greater than one cortical clinical feature. Unfortunately, DP-b99 showed no evidence of efficacy in the treatment of patients with acute ischemic stroke [94].

Why have trials of neuroprotective agents failed?

As previously mentioned in the introductory section of this review, there are a number of reasons why neuroprotective agents have failed in acute ischemic stroke. Effectively translating positive results of neuroprotective therapies from animal studies into the clinical setting has been challenging [95]. Such discordant results between animal studies in ischemic stroke and clinical trials in patients has raised questions of whether the animal models of stroke, in particular rodent models, accurately reflect what is occurring in humans.

First, in preclinical studies, researchers usually apply young and healthy animals. Stroke patients are commonly old with multiple medical comorbidities, such as hypertension, diabetes and hyperlipidemia. Future studies in aging animals with cardiovascular risk factors are warranted.

Second, drug dose is another issue to be considered. Adequate dose–response studies are not routinely carried out in animal models, and also rarely in Phase II stroke trials. Third, in many animal studies, neuroprotective agents were given prior to or shortly after the onset of ischemic stroke. Furthermore, the therapeutic time window in clinical trials has been up to 24 h after symptom onset, and a delayed treatment window limits beneficial effects of neuroprotective agents. Fourth, in most animal studies, efficacy of neuroprotective agents has been evaluated by measuring infarct size, and less frequently by measuring functional outcomes, which is a substantial difference compared with human stroke trials. Standard measures in stroke clinical studies oftentimes include neurologic impairment (NIH Stroke Scale) and a measure of global outcome, the modified Rankin score, and sometimes magnetic resonance imaging outcomes are included, but not routinely [96].

Almost all previous neuroprotective agent studies targeted a specific pathway of acute ischemic stroke. The pathophysiology of acute stroke is complex involving multiple neurobiological processes and pathways. A combined therapeutic strategy (e.g., a 'cocktail' approach of neuroprotective agents targeting multiple pathways of the ischemic cascade) might be more rewarding in patients with acute stroke and, thus, combinations of multiple drugs may be necessary to achieve success with neuroprotectants with or without coadministration of tPA.

Future perspective

The opportunity for neuroprotective agents in stroke remains plausible. The neuroprotection in cerebral ischemia resulting in improvement of functional outcome is supported by preclinical data as mentioned above in our discussion of different neuroprotective agents including free-radical scavengers and antioxidants, NMDA receptor antagonists, inflammatory cascade inhibitors, and different ion-channel blockers/modulators and chelators. Different reasons for the failure of neuroprotective agents in acute ischemic stroke have also been addressed above in detail. The Stroke Therapy Academic Industry Roundtable recommended standards in future preclinical neuroprotective development [95,97–99]. In general, parameters of studying an ideal neuroprotective agent should include:

- Efficacy in both permanent and transient models of ischemic stroke;

- Benefits in at least two different species and in at least two separate laboratories;
- Pathological changes such as infarct volume, as well as functional short- and long-term outcomes;
- An adequate dose–response curve with corresponding serum levels defining the minimal effective and maximal tolerated doses;
- Administrating time window of efficacy;
- Adequate physiological monitoring;
- Blinded evaluations and data analyses.

Future successful development of stroke neuroprotrective drugs will require innovative concepts for preclinical testing and unique approaches based on a proper understanding of different underlying molecular mechanisms and pathophysiology of ischemic stroke. Therefore, the potential for new neuroprotective agents is viable if we can carefully design and conduct preclinical studies and clinical developmental programs with novel approaches to determine efficacy as recommended by the Stroke Therapeutic Academic Industry Roundtable and others, including a stepwise approach starting from key basic steps such as demonstration of an effect in human cell cultures [100,101]. It is worth noting that meeting the Stroke Therapeutic Academic Industry Roundtable criteria does not guarantee transitional success clinically, but we believe that it does decrease the likelihood of failure.

Financial & competing interests disclosure

PB Gorelick serves on the Steering Committees for the IMPACT-24 (Brainsgate) and MACSI (D-Pharm) trials, and as Co-Director of the US DIAS 4 Clinical Coordinating Center (Lundbeck). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed. No writing assistance was utilized in the production of this manuscript.

Executive summary

Background

- Stroke is the largest single cause of adult disability and the second most common cause of death in developed countries.
- Tissue plasminogen activator is the only US FDA-approved drug therapy for acute ischemic stroke patients.
- There is a substantial need for additional effective and safe treatments that will benefit a large number of acute stroke patients.

Neuroprotective agents in acute ischemic stroke

- Many neuroprotective agents have been studied in preclinical stroke research, many with promising results.
- The authors discussed different neuroprotective agents that have been tested in acute ischemic stroke including free-radical scavengers and antioxidants, NMDA receptor antagonists, inflammatory cascade inhibitors, different ion channel blockers/modulators and chelators.

Why have trials of neuroprotective agents failed?

- Translation of most of these neuroprotective drugs has failed in the clinical setting.
- There are different possible reasons that have led to failure of these drugs in the clinical settings including, but not limited to, differences in techniques and end points used in different animal models and clinical trials.
- The other important factors are missing the appropriate early time window for efficacy and salvage of brain tissue, inappropriate patient selection, lack of penetration of study drugs into target tissues and lack of establishment of reperfusion.

Future perspective

- The window of opportunity for neuroprotectants is being revisited as several recent clinical trials show safety and efficacy promise with these agents.

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- ▪ of interest
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