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Small molecule inhibitors in the treatment of cerebral ischemia

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Abstract

Introduction: Stroke is the world's second leading cause of death. Although recombinant tissue plasminogen activator is an effective treatment for cerebral ischemia, its limitations and ischemic stroke's complex pathophysiology dictate an increased need for the development of new therapeutic interventions. Small molecule inhibitors (SMIs) have the potential to be used as novel therapeutic modalities for stroke, since many preclinical and clinical trials have established their neuroprotective capabilities.

Areas covered: This paper provides a summary of the pathophysiology of stroke as well as clinical and preclinical evaluations of SMIs as therapeutic interventions for cerebral ischemia. Cerebral ischemia is broken down into four mechanisms in this article: thrombosis, ischemic insult, mitochondrial injury and immune response. Insight is provided into preclinical and current clinical assessments of SMIs targeting each mechanism as well as a summary of reported results.

Expert opinion: Many studies demonstrated that pre- or post-treatment with certain SMIs significantly ameliorated adverse effects from stroke. Although some of these promising SMIs moved on to clinical trials, they generally failed, possibly due to the poor translation of preclinical to clinical experiments. Yet, there are many steps being taken to improve the quality of experimental research and translation to clinical trials.

Keywords

antagonist; cerebral ischemia; clinical trials; hypoxia; inflammatory cascade; inhibit; ischemic cascade; middle cerebral artery occlusion; mitochondrial injury; preclinical experiments; small molecule inhibitor; stroke; thrombin

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1. Introduction

Stroke is the world's second leading cause of death. Ischemic stroke is characterized by insufficient blood flow to the brain, decreasing the supply of oxygen and nutrients necessary to sustain ATP levels to meet the brain's high metabolic demand. Ischemia-induced energy depletion results in a series of destructive cascades that lead to neuronal death and sensorimotor impairment [1]. There are two types of brain ischemia: focal ischemia is isolated to a specific region, while global ischemia is widespread throughout multiple areas. Brain ischemia is commonly caused by sickle cell anemia, obstructed blood vessels, low blood pressure, heart defects, ventricular tachycardia and blood clots [1–3]. Because of the pathophysiological complexity of ischemic stroke, developing an effective treatment is extremely difficult [4].

Currently, the only FDA-approved clinical treatment for cerebral ischemia is thrombolysis by local or systemic administration of recombinant tissue plasminogen activator (rt-PA) [5]. Rt-PA, however, has significant drawbacks, including a 3 – 4.5 h treatment window after ischemic stroke, which makes a quick and accurate diagnosis crucial [6]. Rt-PA administration after this 3.5 h treatment window may potentially result in numerous well-documented adverse effects, particularly hemorrhagic transformation or even death [7]. Also, the ECASS III clinical trials demonstrated that rt-PA administration after 3 – 4.5 h after stroke onset significantly improved clinical outcomes in stroke patients, but rt-PA was frequently associated with intracranial hemorrhage [8]. Given rt-PA's significant limitations concerning the pathophysiological complexity of cerebral ischemia and its adverse side effects, there is a dire need for new therapeutic interventions.

Due to their membrane-permeable capabilities and specificity for relevant proteins or processes, many small molecule inhibitors (SMIs) have been identified, characterized and critically evaluated in neuropathological injuries and diseases [9]. SMIs are considered to be organic compounds with a low molecular weight of fewer than 900 Da (900 g/mol) [10]. Utilizing a growing understanding of stroke pathophysiological pathways, SMIs have been developed to interfere with the ischemic cascade, ameliorate mitochondrial injury and attenuate the inflammatory response [6]. They have posed as potentially beneficial therapeutic agents and may have a future in stroke treatment (Table 1). However, despite promising results in animal studies, successful translation from bench to bedside of many SMIs' neuroprotective capabilities often failed in Phase II, III or IV clinical trials, possibly on account of weaknesses in selected stroke models, treatment time windows, gaps in knowledge of stroke pathophysiology, evaluated outcomes, model success criteria, infarction area and/or physiological monitoring [11,12]. Yet, many clinical trials evaluating SMIs for ischemic stroke are also still in progress (Table 2).

This review will examine the application of SMIs for attenuating thrombosis, ischemia-induced excitotoxicity, mitochondrial injury and inflammation. The SMIs were chosen based on their specific therapeutic targets (thrombosis, ischemic, mitochondria injury, inflammation, immune-mediated). The selection best summarizes SMIs' potential therapeutic approaches for stroke. To better understand how these SMIs affect stroke pathophysiology, a summary of the ischemic cascade and consequent mitochondrial injury

and inflammatory/ immune response will first be provided. Subsequently, the mechanisms of action of specific SMIs will be discussed, and those SMIs currently undergoing clinical trials and preclinical experimentation will be highlighted. Finally, the therapeutic application of these SMIs for treating ischemic stroke will be assessed and their pitfalls as well as future prospects addressed.

2. Thrombosis

2.1 Coagulation cascade: thrombotic diseases

A majority of strokes are caused by thrombotic or embolic vessel occlusions, making the coagulation cascade one of the main targets for stroke prevention. The coagulation cascade plays an important role in hemostasis (the initial stages of wound healing), the process that stops blood loss after a vessel has been damaged through the accumulation of platelets and fibrin-containing clots. The cascade contains three pathways: the intrinsic, extrinsic and common pathways.

The intrinsic pathway, also known as the contact activation pathway, occurs after trauma or when blood is exposed to collagen in a damaged vessel wall. This pathway is generally made up of many coagulation factors, such as Factors IX, IXa, XI, XIa, XII, XIIa and serine proteases (kallikrein and prekallikrein). In contrast, the extrinsic pathway is activated when tissue is damaged and blood is exposed to tissue factor, which is made up of two coagulation factors (VII and VIIa). Both pathways converge to the common pathway, when Factor X is converted into active Xa. This path is generally made up of various coagulation factors (including Factors II, Va, X, Xa, XIII, XIIIa), thrombin, fibrinogen, fibrin monomers and polymers. Many of the factors involved in this cascade are under evaluation because their inhibition may result in the production of a novel stroke therapeutic. For a more in-depth review of the coagulation cascade and its factors, see reviews by Jerry and Lefkowitz [13], Sinauridze *et al.* [14].

Arteriosclerotic vascular disease, also known as atherosclerosis, is one of the most common vascular occlusive risk factors that lead to thrombotic stroke. Vessel occlusion due to atherosclerosis accounts for 85% of all strokes, making this a traditional target for therapeutic intervention in many preclinical and clinical trials [15]. Atherosclerotic occlusion is caused by the accumulation of fatty materials, such as cholesterol and triglycerides, macrophages, Ca^{2+} and cellular debris, which create atherosclerotic plaque and cause artery walls to harden. Atherosclerotic plaque formation leads to a variety of pathological changes, including ulcerations, thrombosis, calcifications and intra-plaque hemorrhage [16]. Plaque structure must be stable in composition to fully occlude the artery to cause disruption and/or ulceration, a process that occurs over a long period of time [17]. Vessel rupture induces platelet activation and the coagulation cascade system, causing further endothelial disruption [18]. As a result, many destructive vasoactive enzymes are activated, leading to platelet adherence and aggregation to the vascular wall. This adherence and aggregation cause small platelet and fibrin nidi and consequent thrombosis, further occluding the artery and inducing stroke [15,19].

Various other pathological conditions also produce clots and occlude vessels, such as hypercoagulable disorders, fibromuscular dysplasia, arteritis and dissection of a vessel wall. Lacunar infarcts account for 10 – 30% of all strokes and also result from thrombosis. They are caused by the occlusion of deep penetrating arteries and are commonly observed in patients with pre-existing hypertension and diabetes mellitus. Ultimately, thrombosis from atherosclerosis and other pathological conditions leads to the obstruction of blood flow and consequent hypoxia, resulting in anoxia and cerebral ischemia [20].

Atrial fibrillation (AF) is another leading cause of stroke in western populations and is the most common form of arrhythmia in older adults [21]. The irregular beating of the heart can be transient or permanent and may last from minutes to hours or for the rest of the patient's life, depending on the severity of the condition. While AF is the cause of 25% of embolic strokes, the mechanism is still not truly understood. It is thought that clots form inside the heart, due to the rapid irregular beating, whereby these clots travel into arteries and block the blood supply to the brain [22]. The classical treatment for AF has been a vitamin K antagonist, which is an anti-coagulant that decreases the risk of stroke by 60%. Although vitamin K antagonists significantly reduced stroke incidences, they had many drawbacks, including a narrow efficacy window, patients' unpredictable responses to treatment, the need for constant monitoring of coagulation, slow onset and offset of action and constant dose adjustments. Due to the drugs' lack of effectiveness and constant monitoring, new methods were developed to help treat AF and prevent stroke, such as oral anti-coagulant agents that directly inhibit coagulation factors [23].

2.2 Clinical trials

2.2.1 Anti-coagulants—Intravenously administered rt-PA is a thrombolytic that reduces permanent disability and improves patient outcomes after ischemic stroke [24,25]. It is also the only FDA-approved drug for ischemic stroke treatment within the first 3 and 4.5 h after the onset of symptoms [8,26]. Alternative anti-coagulant and thrombolytic agents with improved half-lives, higher target specificities and better safety profiles may potentially be more beneficial than rt-PA and classical anti-coagulants, such as heparin and vitamin K antagonists. SMIs are able to interact with the coagulation cascade in various and effective ways (Figure 1), making them potentially more effective anti-coagulants for stroke prevention and better alternative thrombolytic agents to rt-PA for post-stroke treatment.

In the early 2000s, Ximelagatran, the first oral direct thrombin inhibitor, was developed. While Ximelagatran was shown to have anti-thrombotic efficacy and safety when compared to Warfarin both preclinically and clinically [27], long-term use resulted in liver toxicity. Ximelagatran failed to receive approval in the United States and was eventually taken off the market in Europe [28]. Since this first anti-coagulant failed, Dabigatran was developed and became the second in its drug class to be approved in many countries, due to its effectiveness. Dabigatran is a highly selective and effective direct thrombin inhibitor that is orally administered. In contrast to Ximelagatran, a 2009 clinical trial on the 6-month and 3-year effects of Dabigatran administration reported all the benefits of anti-thrombotic efficacy without any evidence of liver toxicity. This SMI successfully prevented stroke incidence in patients with AF when compared to vitamin K antagonists and has proven to be more

effective with reduced side-effects, including fewer bleeding complications at a lower dose and greater efficacy at a higher dose [29]. In a Phase III clinical trial, called RE-LY, two doses (150 and 110 mg b.i.d.) of Dabigatran were evaluated for efficacy and safety relative to Warfarin in patients with AF. Dabigatran was non-inferior at the lower dose when compared to Warfarin for the primary efficacy endpoint of stroke or systematic embolization, but it was more effective at a higher dose. Both doses of Dabigatran had a decreased incidence of intracranial hemorrhage when compared to Warfarin [30]. Yet, only the lower dose of Dabigatran was associated with a decrease in major bleeding [31]. These clinical trials have demonstrated that Dabigatran is a better alternative to Ximelagatran, without any evidence of liver toxicity. Although Dabigatran has been shown to be more beneficial than its predecessor, its efficacy has not been fully evaluated and it has yet to be approved by the FDA. Several clinical studies are currently recruiting or ongoing to evaluate the efficacy of Dabigatran for treating stroke [32,33].

Another potential oral anti-coagulant that is currently undergoing clinical trials is Rivaroxaban. Rivaroxaban is a direct competitive Factor Xa inhibitor that limits thrombin generation in a dose-dependent manner. A significant advantage of using Rivaroxaban is its consistent anti-coagulation characteristic across a spectrum of patients (of differing age, race, gender, weight), resulting in a dose that does not have to be constantly readjusted, like many other drugs [34]. In a randomized Phase III clinical trial, ROCKET AF, researchers found no significant differences in the efficacy and safety of Rivaroxaban when compared to Warfarin, with the patients showing similar outcomes. The ROCKET AF clinical trials, however, had more patients with advanced disease factors (congestive heart failure, hypertension, age ≥ 75 years, diabetes mellitus, prior stroke or transient ischemic attack or thromboembolism) than in other clinical trials [35,36]. Rivaroxaban, like many anti-coagulants, has significant side-effects, including an increase in blood loss. Much like Dabigatran, there is still a need to evaluate its efficacy and safety. Currently, there are several clinical studies evaluating Rivaroxaban as a preventative stroke treatment in patients with AF that are active and recruiting [37,38].

Apixaban is another oral anti-coagulant treatment given for stroke prevention. This therapeutic is a selective and reversible inhibitor of free or clot-bound factor Xa [39]. Two major clinical trials have been conducted to evaluate the efficacy and safety of Apixaban for the prevention of stroke. The AVER-ROS trial is a comparative study using Apixaban and aspirin to prevent stroke in patients with AF. Although major bleeding and mortality were not significantly different from aspirin-treated groups, Apixaban was shown to be associated with a 55% reduction in the primary endpoints of stroke or systematic embolism over a mean follow-up of 1.1 years [40]. The second major clinical trial assessing Apixaban, ARISTOTLE, was designed to evaluate the efficacy and safety of Apixaban in comparison to Warfarin in patients with AF. The results were positive, as Apixaban-treated patients showed a 21% percent decrease in stroke or systematic embolism, 31% reduction in major bleeding and an 11% reduction in mortality [41]. This SMI demonstrates a high efficacy and positive safety profile, making this drug a potential therapeutic for stroke prevention in patients with AF. Recently, the US FDA has approved Apixaban as a treatment in the prevention of stroke and blood clots in patients with non-valvular AF [42].

2.2.2 Combinational treatment—Many recent experiments have focused on combinational therapeutic agents as treatments for stroke, since these treatments focus on multiple aspects of the injury. As discussed previously, while rt-PA is one of the few FDA-approved treatments for stroke, the fact that 30 – 40% of occluded major vessels were recanalized in the first hour after rt-PA administration, combined with its large reperfusion risk, including intracerebral hemorrhage (ICH), undermines its efficacy [43]. Instead, many agents that can enhance thrombolysis, such as glycoprotein (GP) IIb/IIIa inhibitors, have been under experimentation. GPIIb/IIIa inhibitors are commonly used to attenuate the final common pathway of platelet aggregation, and both clinical and experimental studies have indicated combinational treatment with fibrinolytic agents increases their efficacy in ischemic stroke treatment [44]. Combinational treatments of a fibrinolytic agent and a GPIIb/IIIa inhibitor in animal models of stroke decreased infarct volume, reduced reperfusion deficits and significantly enhanced cortical perfusion when compared to treated groups only given a fibrinolytic agent [45–47].

Eptifibatide, a cyclic heptapeptide, is a GPIIb/IIIa inhibitor and is used to keep platelets in the blood from coagulating. It is currently used for the treatment of acute coronary syndrome. Clinical trials have shown that Eptifibatide, when given alone, was able to reduce the effects of ischemic events and arterial re-occlusions in patients undergoing percutaneous coronary interventions [48–50]. The drug has also not been found to be associated with an increase in ICH. In 2008, the University of Cincinnati College of Medicine conducted a clinical study to evaluate the safety of a combinational treatment using rt-PA and Eptifibatide within 3 h of stroke. It found that patients who were given the combined treatment developed a lower rate of hemorrhagic transformation than those who were only given rt-PA. However, like many other clinical trials, this study had a few disparities between the combination treatment and control groups concerning age and baseline NIHSS scores that would require further testing to evaluate the treatment's efficacy and safety. Despite such disparities, this study's combinational approach as a form of treatment provided two simultaneous mechanistic thrombolytic targets; one through the disruption of the clot's fibrin meshwork and the other through the disaggregation of platelets, thus improving artery reperfusion and reducing side-effects due to rt-PA [51].

2.3 Preclinical experiments

2.3.1 Anti-coagulants—Thrombi formation consists of many coagulation factors, making these targets for therapeutic intervention. BMS-262084, a 4-carboxy-2-azetidinone, is a selective irreversible inhibitor of Factor XIa (FXIa). As discussed previously, FXIa is part of the intrinsic pathway of the coagulation cascade, and its inhibition has had positive outcomes in animal stroke models [52,53]. In a recent study, BMS-262084's efficacy was evaluated in an *in vitro* coagulation model as well as in *in vivo* rabbit models of arteriovenous-shunt thrombosis (AST), venous thrombosis (VT), arterial thrombosis (AT) and cuticle bleeding (BT). All three models (AST, VT and AT) showed the inhibitor's effective anti-coagulant capabilities in decreasing thrombi formation, a result which corroborated with many other studies using similar inhibitors. In addition, a BT model showed a lower bleeding liability in comparison to other anti-coagulants when treated with the FXIa inhibitor. BMS-262084 was demonstrated to be an effective inhibitor by preventing

arterial and venous thrombosis in rabbits. It also had reduced bleeding risk, making this drug an ideal therapeutic agent to prevent clotting that could lead to cerebral ischemia [54]. Although BMS-262084 has shown positive outcomes, more testing needs to be conducted to weigh the effectiveness of the drug as well as its safety and efficacy in humans.

3. Cerebral ischemic cascade

3.1 Pathophysiology of ischemia

A normal supply of oxygen to the brain is vital to generate energy in the form of ATP and meet the brain's high metabolic demands [1]. In ischemic stroke, blood flow is interrupted, leading to a deficient oxygen supply. The loss of oxygen and metabolic substrates for aerobic metabolism, the brain's inability to switch to anaerobic metabolism and the lack of long-term energy supplies result in a severe ATP deficiency [55]. Without ATP, ion pumps, particularly the Na^+/K^+ ATPase, cannot function in maintaining a normal electro-chemical gradient, causing sustained depolarization of glia and neurons. The opening of voltage-gated Ca^{2+} channels from sustained membrane depolarization and insufficient Ca^{2+} pump activity from ATP deficiency lead to increased intracellular Ca^{2+} levels. High intracellular Ca^{2+} induces the release of excitatory amino acid neurotransmitters, particularly glutamate, into the extracellular space. ATP deficiency also results in an insufficient presynaptic reuptake of excitatory amino acid neurotransmitters, causing excess glutamate in the extracellular space. Glutamate binds to ionotropic glutamate receptors (iGluRs), especially NMDA and AMPA receptors, resulting in a massive Ca^{2+} influx and consequent excitotoxicity [56]. Further Ca^{2+} influx induces activation of phospholipases and proteases as well as generation of free radicals and reactive oxygen species (ROS). Phospholipases degrade the cell membrane, allowing water to cross into the cell. Furthermore, due to glutamate-mediated over-stimulation, Na^+ and Cl^- enter neurons through monovalent ion channels, increasing cellular toxicity and driving passive flow of water into the cell [57]. The water influx results in cell swelling, shrinking of the extracellular space and cytotoxic edema, leading to necrosis. Through this pathophysiology, small molecules could potentially interact with the ischemic cascade in various ways to inhibit and attenuate cerebral ischemic injury (Figure 2).

3.2 Clinical trials

3.2.1 NMDA antagonist – glycine-binding site—Typically, NMDA receptors are inhibited at their glutamate-binding site. Unlike most NMDA inhibitors, GV150526 antagonizes NMDA receptors at their glycine-binding site. Inhibiting this alternative-binding site has had beneficial advantages in several animal models, including fewer negative side effects [58,59]. In permanent and transient middle cerebral artery occlusion (MCAO) experimental models, the alternative inhibitor reduced infarct volume after both pre- and post-treatment regimens [60,61]. In 1999, a tolerance clinical trial was conducted and suggested GV150526 was well received at multiple doses in patients with cerebral ischemia [62]. In a Phase II clinical trial, researchers further evaluated multiple doses 12 h after ischemic stroke. GV150526 also had an excellent preliminary safety profile in ischemic stroke patients with only mild accompanying adverse side effects. Unfortunately, this study did not show statistical significance and could not report GV150526 effects on ischemic

stroke patients [63]. Currently, there are no clinical trials evaluating this SMI's neuroprotective attributes in human patients.

3.2.2 Ca²⁺ channel inhibitors—Since Ca²⁺ plays an integral role in the ischemic cascade, inhibiting Ca²⁺ influx is a main focus for ischemic neuroprotection [64]. Magnesium, considered 'nature's calcium blocker', is a bivalent molecule that competes with Ca²⁺ for Ca²⁺ receptor binding or passage through an ion channel [65,66]. In cerebral ischemia experimental models, magnesium prevented Ca²⁺ influx and consequent excitatory amino acid release in neurons by inhibiting Ca²⁺ channels, decreasing Ca²⁺ passage through the NMDA-receptor channel and reducing mitochondrial injury. In a permanent focal ischemia model, magnesium was given at multiple time points after injury, improving survival rates as well as neurological outcomes [67]. A clinical trial was launched in 1984 to evaluate magnesium tolerance in human stroke [68]. Several other clinical trials began dose regimens but failed due to small sample sizes, short treatment windows or toxic doses [69–71]. Failed experimental to clinical translation may be due to inaccurate or insufficient preclinical data. Other explanations for this failed clinical trial may lie in the insufficient bioactivity of the molecule, which did not respond as expected in patients with stroke. Currently, UCLA is directing a Phase III clinical trial to evaluate the effectiveness and safety of field-initiated magnesium sulfate for improving long-term functional outcomes of acute stroke patients and found that magnesium in patients with acute stroke is safe and feasible, yet the study is ongoing and recruiting patients, so a conclusion on its efficacy has not been determined [72,73].

Nimodipine, a Ca²⁺ channel inhibitor, dilates cerebral blood vessels and readily passes the BBB while improving cerebral blood flow and attenuating necrosis and apoptosis in animal models of neurodegenerative diseases, like dementia and cerebral ischemia [74–78]. In a preclinical dementia model, Nimodipine treatment significantly improved cognitive function and reduced pro-inflammatory cytokine levels in the hippocampus, including NF- κ B, TNF- α and IL-1 β [78]. In a global cerebral ischemia animal model, Nimodipine inhibited Ca²⁺ ion channels, decreasing glutamate release. Choi *et al.* used electroencephalography electrodes to measure glutamate release and reported that Nimodipine-treated groups had significantly decreased glutamate release after ischemia and reperfusion injury [79]. Phase III clinical trials (VENUS) evaluating Nimodipine for ischemic stroke treatment have been completed. Nimodipine was orally administered within 6 h after stroke and given 10 days consecutively, but the trial showed no significant difference between the delayed treatment and placebo groups [80]. Currently, a Phase IV clinical trial (NICE) is in progress to assess early Nimodipine treatment after acute ischemic stroke in an effort to attenuate cognitive impairments. Data from this trial, however, has not been publicly disclosed [81]. Recently, Nymalize, an oral Nimodipine derivative, has been FDA approved to treat patients with specific cerebral hemorrhage, demonstrating this SMI's potential to treat cerebral ischemia in the future [82].

3.3 Preclinical experiments

3.3.1 NMDA antagonists – glutamate binding site—In the ischemic cascade, the over-stimulation of NMDA receptors plays a crucial role in excitotoxic processes, and its

inhibition has been a popular therapeutic target in ischemic stroke research. NMDA receptor antagonism is neuroprotective in neurodegenerative disease [83]. Kynurenic acid is a well-established NMDA inhibitor with neuromodulatory and neuroprotective properties [84–86]. This SMI, however, poorly penetrates the BBB, leading to the development of a brain penetrant derivative, 2-(2-*N,N*-dimethylaminoethylamine-1-carbonyl)-1*H*-quinolin-4–1 hydrochloride, which selectively inhibits the NR2B subunit of NMDA receptors [87]. This SMI has been shown to antagonize NMDA receptors in migraine animal models, inducing nNOS production [88]. In a recent study using a global cerebral forebrain ischemia animal model, pre- and post-ischemia treatment using the Kynurenic acid analog (2-(2-*N,N*-dimethylaminoethylamine-1-carbonyl)-1*H*-quinolin-4-one hydrochloride) significantly diminished hippocampal CA1 cell loss and preserved LTP expression at the Schaffer collateral-CA1 synapses [89]. Unlike its predecessor, the analog has been shown to easily cross the BBB and have potent neuroprotective properties that make it a potentially clinically relevant therapeutic agent for ischemic stroke. Yet, little experimentation has been done to evaluate the efficacy and safety of this drug in animal models, making it difficult to determine whether the drug can be used in a clinical setting.

Memantine is a low-affinity NMDA glutamate receptor antagonist with fast channel unblocking kinetics, preventing glutamate from occupying the channels and interfering with normal synaptic transmissions [90]. It is one of the few FDA-approved drugs that successfully inhibits glutamate-induced NMDA receptors in humans and is commonly used as a therapeutic agent for Alzheimer's disease and dementia [91]. Current clinical trials are testing this drug as an intervention for aphasia after stroke, yet its use in cerebral ischemia has not been clinically determined. This SMI has neuroprotective effects in experimental models of neurodegenerative disease [91]. Previous studies have suggested Memantine is neuroprotective in ischemic stroke models and was strongly associated with NMDA inhibition [92,93]. Memantine has the potential to become a neuroprotective therapeutic agent for cerebral, yet much more experimentation needs to be conducted to fully evaluate Memantine's capabilities.

NMDA stimulation also leads to the over-expression of dopamine, causing neurotoxicity. This mechanism has been well studied in cerebral ischemia models as well as evaluated in clinical trials [94–97]. The abundance of dopamine, much like glutamate, generates free radicals, causing neuronal damage. Thus, dopamine antagonism is a therapeutic target [98]. MK-801 is a potent and selective non-competitive NMDA glutamate receptor antagonist that blocks the opening of NMDA receptor-operated ion channels. While MK-801 failed in clinical trials during the 1980s, it is still being evaluated in preclinical models to further elucidate stroke's pathophysiology and uncover novel therapeutic mechanisms. MK-801 is a neuroprotective agent in hypoxia and stroke experimental models [99–101]. MK-801 improves functional recovery, reduces histological damage, decreases oxidative stress and increases the survival rate of ischemic animals [102–104]. Furthermore, this SMI reduced dopamine expression in a recent *in-vitro* hypoxia study. Additionally, another study measured dopamine activity using a voltammogram and found significantly decreased dopamine levels in MK-801-treated groups [105]. Despite having beneficial outcomes, MK-801 revival to a clinical setting is highly unlikely, as new therapeutics have been developed that are known to be more effective and translatable towards a clinical setting.

4. Mitochondrial injury

4.1 Pathophysiology of mitochondrial injury

Mitochondria serve as the ‘cellular power house’ for neurons and most other cell types. They are responsible for the production of ATP by oxidative phosphorylation and also play a key role in vital signal transduction pathways (i.e., buffering and storage of intracellular Ca^{2+}), control of the cell cycle and programmed cell death (apoptosis) [106]. During cerebral ischemia, inadequate oxygen and nutrient supplies cause mitochondrial dysfunction, inducing neuronal death after injury [107,108]. This section will summarize ischemia-induced mitochondria injury and consequent necrosis and apoptosis and will describe specific SMIs that ameliorate mitochondrial injury.

During ischemia, the brain is deprived of oxygen and glucose, leading to ATP deficiency [1]. This leads to excitotoxic neurotransmissions, causing increased Ca^{2+} conductance across the neuronal plasma membrane through ion pumps [109]. The rise in intracellular Ca^{2+} adversely affects neuronal survival by destabilizing the mitochondria membrane potential (Mmp), which is vital for ATP synthesis through Ca^{2+} sequestration from the cytosol into the mitochondrial matrix and for the generation of ROS to temper oxidative stress. Mmp destabilization causes mitochondria permeability transition (MPT), a process where increased mitochondria permeability leads to mitochondrial swelling and eventual membrane rupture, causing release of free radicals that increase endoplasmic reticulum stress [109–111]. The MPT is mediated by the MPT pore (MPTP), a high conductance channel between the outer and inner membranes, and the MPTP opening plays a key role in necrosis and apoptosis after ischemia [112,113]. The opening of the MPTP induces Ca^{2+} uptake and ROS production, inhibition of ATP synthesis and release of cytochrome C [114]. Cytochrome C release also results from mitochondrial outer membrane permeabilization (MOMP), which transmits apoptotic signals [115]. Cytochrome C, a pro-apoptotic small heme protein, co-activates caspase-8 and caspase-3 in conjunction with the Smac/DIABLO protein complex, inducing cell death [116]. SMIs have the potential to inhibit the pathophysiology of mitochondrial injury in an effort to preserve cells and mitochondria potential, attenuating brain injury after stroke (Figure 3).

4.2 Clinical trials

4.2.1 Anti-oxidants—Edaravone (Edv, 3-methyl-1-phenyl-2-pyrazolin-5-one) is an SMI with anti-oxidative and anti-apoptotic characteristics. This SMI ameliorates neurodegenerative disease and injury, such as cerebral ischemia, traumatic brain injury and Parkinson’s disease in animal models [117–119]. In an MCAO model, daily treatment of Edaravone for 14 days starting immediately after ischemic insult significantly attenuated neurological deficits, improved motor function and suppressed oxidative stress by 18.1% compared to the single-treated and non-treated groups [117]. Due to promising results in preclinical models using this SMI, Japan conducted several clinical trials. One comparative Phase IV clinical trial compared two drugs, Edaravone and Sodium Ozagrel, but clinical outcomes have not yet been published. In another Phase IV clinical trial conducted in Japan, researchers evaluated a combinational therapy of two Japanese approved drugs, Edaravone and Argatroban. Much like the comparative trial, this study has not disclosed experimental

outcomes [120]. In a recent study, ECCS-AIS clinical trials demonstrated that patients treated with Edaravone were associated with better neurological outcomes at 3 months [121]. There are currently active studies evaluating Edaravone for stroke treatment [122]. Due to the lack of clinical data, Edaravone efficacy comes into question. Although this drug is highly effective in animal models of stroke, it has yet to be fully evaluated in a clinical environment. Despite the lack of clinical data, Edaravone is currently approved for treatment of acute ischemic stroke in Japan.

NXY-059 has free radical scavenging properties and inhibits the inflammatory response. This anti-oxidant was neuroprotective in animal models of cerebral ischemia and traumatic brain injury [123–126]. In an animal model of focal cerebral ischemia, NXY-059 treatment reduced infarct volume from 37.2 to 12.5% and inhibited cytochrome C release [126]. A meta-analysis evaluated preclinical experiments of transient and permanent MCAO using NXY-059 treatment and reported decreased infarct volume by 42%. The meta-analysis, however, concluded that the efficacy of this drug is questionable due to the low quality of preclinical studies that tested it, thus the analytical results should be taken with caution [123,124]. NXY-059's therapeutic effectiveness for stroke was evaluated in two clinical trials: SAINT II and I. SAINT I was a Phase III clinical trial (a double blinded, randomized, placebo-controlled trial) that showed significant improvement in disability outcomes at 3 months after stroke, but neurological outcomes were not significantly improved [127]. SAINT II trials also failed to show significantly improved neurological outcomes, indicating that NXY-059 cannot protect against ischemic insult [128]. Much controversy, however, surrounds these clinical trials because of the possibility of trial failure due to poor preclinical translation, trial design, insufficient bioactivity of the molecule, insufficient preclinical data, optimal drug dosage and more, which are all common problems in many evaluations of potential therapeutic interventions [129,130].

4.3 Preclinical experiments

4.3.1 DRP-1 inhibitor—Mitochondria are autonomous and morphologically dynamic organelles that precisely balance ongoing fission and fusion within the cell [131–134]. Mitochondrial fission is caused by the constriction and cleavage of mitochondria by fission proteins, such as dynamin-related protein (Drp-1) and Fission 1, and is linked to apoptosis [135–137]. Drp-1 is a key player in mitochondrial-dependent apoptosis [138]. Mitochondrial division inhibitor (Mdivi-1) selectively inhibits Drp-1 GTPase activity by preventing its self-assembly and GTP hydrolysis. As a result, it blocks MOMP and cytochrome C release, inhibiting apoptotic cell death [139]. A recent article demonstrated mdivi-1 pre-treatment decreased Drp-1 expression, reduced cytochrome C release and diminished apoptosis in an MCAO Wistar rat model [140].

4.3.2 GSK-3 β inhibitor—Glycogen synthase kinase 3 β (GSK-3 β), an enzyme that modulates cell growth and survival, has been targeted for the treatment of diabetes, brain injury and other neurodegenerative diseases [141–143]. It is a key component in the neurodegenerative processes of Alzheimer's disease. Inhibiting this enzyme with lithium and Chir025 was neuroprotective after cerebral ischemia/reperfusion by improving cellular tolerance [141,144]. Published data suggest GSK-3 β inhibition reduces Bax mitochondria

translocation and mitochondria disruption, reducing cytochrome C release as a result [145]. Small molecule 4-benzyl-2-methyl-1,2,4-thiadiazolidine-3,5-dione (TDZD-8) is a highly potent, selective GSK-3 β inhibitor that reduced inflammation and tissue injury after gut ischemia/ reperfusion injury, colitis, spinal cord injury and both endotoxic and hemorrhagic shock [146–148]. In another article, possible mechanistic effects from GSK-3 β inhibition by TDZD-8 were evaluated in a cerebral ischemia/reperfusion animal model. They found TDZD-8 decreased COX-2, iNOS and intracellular adhesion molecule-1(ICAM-1) expression, reduced ROS overproduction and attenuated decreased hippocampal superoxide dismutase (SOD) activity [149].

4.3.3 Anti-oxidants—Since many studies point to oxidative stress from ROS, which is linked to mitochondrial dysfunction, as a main source for neuronal damage after cerebral ischemia, anti-oxidative therapies were developed as potential stroke treatments. In early transient cerebral ischemia, free oxygen radicals cause much brain tissue damage, and anti-oxidant enzymes are inactivated [150]. Due to these adverse effects, pharmacological strategies aimed at ameliorating oxidative damage are considered one of the most promising stroke therapeutic avenues. SMI Genistein (4',5,7-trihydroxyisoflavone) is a nutraceutical molecule extracted from soybean seeds that has potential health benefits for breast and prostate cancers, cardiovascular disease and post-menopausal ailments and is currently in clinical trials for these diseases [151]. The stroke research community is interested in this SMI due to its anti-oxidative properties, which protect against vascular dysfunction by preventing degenerative oxidative modifications, upregulating endogenous anti-oxidant signaling pathways, preventing H₂O₂-induced apoptosis via ER β regulation and Bcl-2/Bax expression, and enhancing cell survival signaling, such as in the PI3K pathway [151,152]. Genistein crosses the BBB and is neuroprotective without appreciable toxic effects in animal models, thus making this SMI a potentially clinically relevant drug for ameliorating cerebral ischemia [151]. Yisong Qian and associates evaluated Genistein in an adult mouse MCAO model and found oral treatment for two consecutive weeks reduced infarct volume and improved neurological deficits. Isoflavone also effectively scavenged the free radicals, inhibiting lipid peroxidation and stimulating anti-oxidative enzyme activities, SOD and GPx [153]. Other studies corroborated their findings in *in vitro* and *in vivo* brain ischemia models [154,155]. Genistein inhibits ischemia-induced neuronal damage and prevents oxidative stress by, in part, inhibiting mitochondria-dependent apoptotic pathways and ROS-induced NF- κ B activation [155]. Thus, Genistein exhibits significant neuroprotective effects during cerebral ischemia/reperfusion injury, and people at risk for occlusive stroke may take it as a dietary supplement to potentially improve neurological outcomes in the event of a stroke. Whether Genistein can successfully translate into the clinical setting for stroke treatment remains unknown and should be approached with caution, as most treatments fail to translate in a clinical setting for multiple reasons. Such disparities that plague translation are insufficient amount of preclinical experimentations to determine the drugs' efficacy, inaccurate results, logistics errors with data and much more.

5. Inflammation and immune response

5.1 Pathophysiology of inflammatory and immune response

The inflammatory cascade plays a key role in modulating the survival and death of cells in the post-ischemic brain. By understanding the inflammatory cascade following ischemic injury, it is possible to evaluate the potential therapeutic effectiveness of specific anti-inflammatory SMIs. Thanks to recent advancements in the cerebral ischemia research field, the integral role of the immune response and inflammatory processes in the ischemic cascade has been more clearly elucidated [156,157]. After ischemic insult, cerebral endothelial and microglia cells are activated from hypoxia, and they increase ROS production and consequent oxidative stress [158,159]. Additionally, adhesion molecule expression, such as intracellular adhesion molecules (ICAMs), vascular adhesion molecules (VCAMs), selectins (P-selectin, E-selectin, L-selectin) and integrins (Mac-1 and LFA-1), is increased on endothelial cells, leukocytes and platelet cell surfaces [157,160]. Concurrently, expression of pro-inflammatory cytokines, proteases and chemokines is mediated through the MAPK signal transduction pathway and the NF- κ B transcription factor, exacerbating the immune response. Pro-inflammatory signals recruit immune cells (T and B lymphocytes, neutrophils, natural killer cells, dendritic cells and macrophages), which adversely damage endothelial cells by activating iNOS and COX-2 pathways [161]. Neutrophils, which are the first to infiltrate the infarcted region, exocytose granules containing pro-inflammatory and degenerative agents (such as NO derived from iNOS, nicotinamide adenine dinucleotide phosphate [NADPH] oxidase-derived ROS and MMPs), resulting in endothelial damage, BBB disruption, hemorrhagic transformation, vasogenic edema, neuronal damage and consequent neurological deficits [160,162]. Although inflammation plays a crucial role in tissue repair, its many detrimental consequences suggest immunomodulatory therapeutic modalities may be beneficial for stroke. Through this pathway, SMIs have the potential to attenuate inflammatory signals and the immune response by inhibiting various mechanisms (Figure 4).

5.2 Clinical trials

5.2.1 Phosphatidylcholine precursor—Citicoline, a key phospholipid in the cell membrane, is an essential precursor for phosphatidylcholine synthesis. This drug has neuroprotective effects in acute ischemic stroke by inhibiting free radical generation, lipid metabolism, fatty acid release and apoptosis and by stabilizing the cell membranes [163–166]. In a preclinical study of transient cerebral ischemia, Citicoline treatment at 0 and 3 h after cerebral ischemia improved neurological deficits and behavioral performance in learning and memory tasks [167]. In a permanent MCAO animal model, Citicoline inhibited the MAPK pathway, suggesting it has anti-inflammatory effects [168]. Although a recent report stated that Citicoline improved neurological, functional and global outcomes in acute ischemic stroke patients [169], the results measured by the planned analyses from a Phase III trial indicated Citicoline 2000 mg/day administered for 6 weeks was safe but ineffective in improving outcomes in acute ischemic stroke patients [170]. In another recent Phase III trial (ICTUS Study: International Citicoline Trial on Acute Stroke), patients received Citicoline within 24 h after the onset of symptoms (1000 mg i.v. every 12 h during the first 3 days and orally thereafter for a total of 6 weeks [2 × 500 mg oral tablets given every 12 h]), and the

results revealed that Citicoline is not an efficacious treatment for moderate-to-severe acute ischemic stroke [171]. Poor translation from experimental stroke models to clinical applications may be due to inaccurate or insufficient preclinical data, insufficient bioactivity of the molecule, trial design and optimal drug dosage. Also, poor communication between different clinical trials, causing two of the three trials to fail, may be a reason for Citicoline's failure.

5.3 Preclinical experiments

5.3.1 PDE4 inhibitor—To date, no effective pharmacological strategy exists that ameliorates BBB disruption and consequent vasogenic edema due to cerebral ischemic insult and successive inflammation [172]. Rolipram is a selective phosphodiesterase-4 (PDE4) SMI, resulting in increased intracellular cAMP levels in many tissues including the brain [173]. Initially, this SMI was developed as an anti-depressant, yet it has anti-inflammatory and BBB stabilizing effects in the CNS [174]. Furthermore, Rolipram treatment after global cerebral ischemia diminished tissue damage and neuronal loss in the hippo-campus, ameliorating memory deficits. Additionally, Rolipram readily crosses the BBB, reaching concentrations twice as high as in plasma, and distinct Rolipram binding sites have been identified in such crucial structures as the hippo-campus after experimental stroke [175–177]. In a recent study, PDE4 inhibitors' role in a transient MCAO model was evaluated. The study demonstrated PDE4 inhibitor treatment 2 h post-stroke decreased infarct volume and improved neurological outcomes. Neutrophil infiltration was also significantly decreased. PDE4 inhibition significantly reduced pro-inflammatory cytokine expression, such as IL-1 β and TNF- α , after cerebral ischemia whereas TGF- β expression increased. This study also demonstrated that Rolipram reduced BBB disruption, inflammation and thrombosis by attenuating pivotal mechanisms within the cerebral ischemic cascade [178]. Although this SMI has many positive attributes, significant adverse side effects need to be overcome, such as gastrointestinal discomfort, hypotension, fear of the side effects or flushing, which forced some patients to discontinue therapy in clinical trials [179,180]. Despite its negative side effects, it is a promising neuroprotective agent for treating cerebral ischemia, yet caution should be exercised in its potential clinical application.

5.3.2 5-LOX inhibitor—Secondary brain injury from ischemia-induced inflammation is a leading cause of brain damage [161,181]. 5-Lipoxygenase (5-LOX) is responsible for oxidizing arachidonic acid into leukotrienes and pro-inflammatory mediators, and its expression increased in rat brains after permanent focal cerebral ischemia [182,183]. More specifically, 5-LOX expression increased in macrophages/microglia after focal cerebral ischemia, and its activation drives inflammatory processes in the ischemic hemisphere, leading to cerebral infarct expansion and worsened neuronal damage [184]. Zileuton is an SMI selective for 5-LOX, is highly effective at preventing leukotriene formation *in vitro*, *ex vivo* and *in vivo*, and is currently used by asthma patients to treat their symptoms [185]. A mechanistic study determined that Zileuton decreased NF- κ B activation, iNOS expression and TNF- α and IL-1 β expression [183]. Xian-Kun Tu and associates investigated Zileuton treatment in focal cerebral ischemia using an MCAO rat model. They demonstrated Zileuton, administered orally, reduced brain edema and decreased 5-LOX expression [182]. Although the exact mechanisms of this SMI are unclear, its demonstrated anti-inflammatory

and neuroprotective effects through 5-LOX inhibition make it a promising therapeutic agent for cerebral ischemia, but further investigation of the effects of this drug are needed.

5.3.3 PARP-1 inhibitor—*N*-(6-oxo-5,6-dihydrophenanthridin-2-yl)-*N,N*-dimethylacetamide (PJ34) is a poly(ADP-ribose) polymerase-1 (PARP-1) inhibitor that attenuates inflammation after cerebral ischemia, improving neurological outcomes [186,187]. PARP-1 drives the inflammatory response and consequent neuronal damage by assisting with pro-inflammatory gene transcription [188,189]. It is a co-activator that facilitates NF- κ B subunit binding to DNA promoter sites and enhances transcriptional complex formation [189]. NF- κ B regulates expression of several pro-inflammatory cytokines and proteases, including iNOS, ICAM and TNF- α [190]. PARP-1 inhibition has been shown to downregulate pro-inflammatory factor expression, suppress NF- κ B-dependent gene transcription in microglia and decrease activated microglia [187]. Delayed treatment of PJ34, given 48 h after cerebral ischemia in rats, reduced microglia activation and astrogliosis, promoted neurogenesis in the hippocampal CA1 and improved spatial memory [191]. Since many stroke patients are not diagnosed within the time window for r-tPA treatment, this SMI may potentially be a clinically relevant treatment.

6. Expert opinion

With a growing understanding of stroke pathophysiological pathways, new perfusion-enhancing compounds and potential neuroprotective drugs have been developed. Due to their membrane-permeable capabilities and specificity for relevant proteins or processes, SMIs could be a promising strategy in stroke therapy. Many SMIs have been developed to inhibit thrombosis, reduce ischemia-induced excitotoxicity, ameliorate mitochondrial injury and attenuate the inflammatory response. Indeed, SMIs specific for different pathophysiological pathways are being widely investigated in both clinical trials and preclinical experiments. As discussed, we reported the beneficial results from many experimental animal models and *in vitro* studies using SMIs. Many studies reported that these SMIs increased survival rate, reduced ischemic insult and decreased neuronal damage. Yet, many of these SMIs are in their infancy and it remains to be seen if they will successfully translate into a clinical application.

SMIs, however, face the same pitfalls as many other drugs currently undergoing preclinical experimentation, including difficulty in translating from bench to bedside. The development of a new therapeutic drug for the treatment of cerebral ischemia is difficult, expensive and relies on extensive experimentation with no guarantee of successful translation into clinical applications [6]. Although basic research continues to identify potential therapeutics, these therapeutics fail to address all aspects of cerebral ischemia, since they strictly focus on particular components of stroke (e.g., inflammation, pain, immune response, etc.). Thus, demonstrating the effectiveness of a drug in some models does not mean that it will be effective in other models. Even when multiple animal models are used to determine the efficacy and safety of a potential therapeutic, firm determinations are difficult due to the differences in species, brain structure, brain composition, tolerance of cerebral ischemia and drug reaction in the animals. Yet, trying to resolve these discrepancies in drug efficacy may provide more insight into the mechanisms of action or help predict outcomes in human

patients. Organizations such as the Stroke Therapy Academic Industry Round Table (STAIR) and the National Institute of Neurological Disorders and Stroke (NINDS) are trying to remedy this issue by providing guides to answer these discrepancies and improve the quality of experimental and clinical research [192,193]. Furthermore, despite the weak translation from experimental to clinical applications, it would be a disservice to not recognize the many laboratories that continue to make advancements in closing the gap.

As experimental therapeutics make their way into clinical usage, many factors cause them to fail. Much of this failure is due to stroke research's primary use of young, male rats, whereas clinical patients are far more complicated due to their concomitant pathologies, such as hypertension, hyperglycemia and diabetes [194]. There are also issues with the efficacy and safety of drugs, which make it difficult to determine the risk/benefit profiles for potential therapeutics. Clinical trials also face challenges in molecule bioactivity, logistics, clinical trial design and optimized dose. For instance, some SMIs have established and pronounced bioactivity in animal models but have not been thoroughly evaluated in human patients. Logistical issues are notorious for causing clinical trials to fail, as programming is still susceptible to errors in randomization codes and labels. As companies rush for a specific drug to be tested into the clinical field, they become vulnerable to errors in proofing their randomization algorithms/labels again, resulting in misleadingly negative outcomes and conclusions. Also data analysis can be inaccurate due to errors in the programming (e.g., the trial's protocol may call for the exclusion of specific patients, but the programmer misses this information in the program, causing an error). One of the most common reasons for a clinical trial to fail is its trial design. Because there are many variables when designing a clinical trial, it is often difficult to design the perfect experiment in a clinical setting. To ensure that a clinical trial is conducted correctly, they need to search for the proper patients that meet their criteria, the optimal dose that ensures there is enough bioactivity to cause an effect and the right clinically relevant endpoints that evaluate the efficacy as well as the safety profile of the therapeutic agent. Finally, many of the aforementioned SMIs may have failed in clinical trials not because of weaknesses in preclinical experiments or trial design and logistics but because of their narrowed targeting on one aspect in one pathophysiological pathway, making these experimental models one dimensional, which primarily focus on one aspect of stroke. Indeed, an SMI's specificity may be both its greatest strength and its weakness. For example, inhibiting NMDA-induced excitotoxicity has established neuroprotective effects in preclinical ischemic stroke models, but NMDA-induced excitotoxicity may only play a small part in the grand scheme of ischemic stroke pathophysiology in the clinic. As we have learned over the years, stroke is a multi-pathophysiological traumatic injury that involves several integrated pathways. Thus inhibiting one pathway may be futile unless other adverse synergistic pathways are inhibited as well. A multi-dimensional therapeutic approach towards ischemic stroke treatment should be taken whereby multi-modal combinatorial treatments utilizing SMIs are investigated. An SMI's strength lies in its specificity, thus therapeutically applying multiple SMIs that precisely inhibits different pathophysiological pathways may work symbiotically to improve ischemic stroke outcomes. Multi-modal, combinatorial treatments, however, should be approached with caution, as questions concerning their efficacy, safety and tolerability in

patients need to be answered first. More preclinical research into these approaches is warranted.

Thanks to advancements in cerebral ischemia research, we understand that stroke is a multi-pathophysiological disorder that includes several integrated pathways leading to injury progression and neuronal damage. Because of stroke's complexity, developing successful clinical interventions is excruciatingly difficult. The majority of experiments focus too much on one SMI for primary and secondary stroke prevention and acute stroke therapy. Thus, a single SMI is limited as a beneficial neuroprotective therapy since it inhibits only a small part of stroke's vast, complex pathophysiology. To some extent, this may explain why clinical translation of some SMIs' neuroprotective benefits is rarely successful, despite their neuroprotective effectiveness in ischemic stroke animal models. Many studies, however, can use multimodal combinatorial therapies that focus on multiple aspects of stroke to enhance treatment efficacy. As discussed previously, treatment using a fibrinolytic agent and GPIIb/IIIa inhibitor simultaneously targets two thrombolytic mechanisms by disrupting the clot's fibrin meshwork and disaggregating platelets, which improved artery reperfusion and had reduced side effects compared to rt-PA. This treatment approach could also reevaluate highly effective drugs that were considered too toxic, since this method may reduce those drugs' side effects. Indeed, future therapeutic strategies for ameliorating cerebral ischemia may involve such combinatorial approaches.

Despite several roadblocks, SMIs demonstrate positive outcomes when used to treat cerebral ischemia. With further evaluation and development, SMIs may potentially be extensively used in the future to inhibit multiple stroke pathways. New approaches, such as multimodal combinatorial therapies, may potentially reduce permanent disability in stroke patients and should be considered for preclinical and clinical assessments.

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Article highlights.

- Cerebral ischemia is the second leading cause of death, and there is a dire need for new interventions.
- With further elucidation of stroke's complex pathophysiology, small molecule inhibitors (SMIs) are being developed to specifically target certain portions of the ischemic cascade.
- New anti-coagulants show potential to become more effective and safer than rt-PA.
- Novel multimodal SMIs are able to interfere with multiple mechanisms in stroke, potentially paving the way for future drug development and therapeutic approaches.
- Weak translation between experimental and clinical trials has resulted in many discrepancies, but recent steps have been made to close the gap between bench and bedside.
- Much like preclinical studies, clinical trials are also vulnerable to study pitfalls such as: logistics, study design and dose optimization to name a few.

Figure 1. Coagulation cascade.

The coagulation cascade is made up of three pathways (color coded). The intrinsic pathway (white portion), also known as contact system, occurs when there is trauma to the blood or blood is exposed to collagen in a damaged vessel wall. The extrinsic pathway (darker gray) is activated when tissue or cells are damaged, exposing blood to tissue factor. Eventually, both pathways converge into the common coagulation pathway (lighter gray), which results in clot formation. Small molecule inhibitors (SMIs) treat ischemic insult by selectively targeting pivotal factors within the cascade.

Indicated by the inhibitory markers; SMIs in bold are used in the text.

Figure 2. Pathophysiology of ischemia.

During the ischemic insult, the brain succumbs to energy failure, causing depolarization of glia and neurons. Depolarization induces Ca²⁺ influx, evoking the release of excitatory amino acids, such as glutamate. Glutamate activates NMDA/AMPA receptors, leading to necrosis and apoptosis. Small molecule inhibitors (SMIs) treat ischemic insult by selectively targeting pivotal factors in ischemic pathophysiology.

Indicated by the inhibitory markers; SMIs in bold are used in the text.

Figure 3. Pathophysiology of mitochondrial injury.

Consequent energy failure from cerebral ischemia results in an over-abundance of Ca^{2+} and reactive oxygen species, causing Mmp depolarization. Depolarization leads to the opening of the mitochondria permeability transition pore, evoking the mitochondria permeability transition (MPT). MPT causes the release of free radicals and Cytochrome C from the outer mitochondrial membrane, resulting in necrosis and apoptosis. Small molecule inhibitors (SMIs) treat ischemic insult by selectively targeting pivotal factors related to mitochondrial injury.

Indicated by the inhibitory markers; SMIs in bold are used in the text.

Figure 4. Pathophysiology of inflammatory and immune response.

After ischemic insult, the immune response increases reactive oxygen species and oxidative stress, causing the upregulation of pro-inflammatory signals. Concurrently, NF-κB and MAPK signaling amplifies the inflammatory response. Pro-inflammatory signals lead to increased MMP activity, evoking further BBB disruption and endothelial cell damage, causing hemorrhagic transformation, vasogenic edema and neurological deficits. Small molecule inhibitors (SMIs) treat ischemic insult by specifically targeting pivotal factors in the immune response.

Indicated by the inhibitory markers; SMIs in bold are used in the text.

Table 1.

Neuroprotective SMIs undergoing preclinical evaluations.

Pathophysiological target	Classification	Agent	Mechanism of action	Ref.
Thrombosis Ischemic cascade	Anti-coagulant NMDA glutamate inhibitor	BMS-262084 Eliprofil	A selective irreversible inhibitor of Factor XIa Selective NMDA glutamate receptor antagonist	[54] [195]
		Kynurenic acid	Non-selective antagonist at NMDA and AMPA	[89]
Mitochondria injury	Anti-oxidant	Memantine	Low-affinity NMDA glutamate receptor antagonist	[196]
		MK-801	Selective non-competitive NMDA glutamate receptor antagonist	[105]
		Genistein	Free radical scavenger, inhibiting lipid peroxidations	[155]
		Daidzin	Selective inhibitor of mitochondrial aldehyde dehydrogenase 2 (ALDH-2)	[197]
		TDZD-8	Downregulated COX-2, iNOS, ICAM-1 and ROS production	[149]
Inflammation and immune response	NOS inhibitor	Mdivi-1	Selective inhibitor of mitochondrial division DRP (dynamin-related GTPase)	[140]
		3-Bromo-7-nitroindazole S-methylisothiourea hemisulfate Salt	A potent inhibitor of all NOS isoforms Potent inhibitor of iNOS	[198] [199]
		BW B70C	Anti-inflammatory regulation of NF- κ , iNOS	[184]
		Zileuton	Decreases NF- κ , iNOS and cytokines (TNF- α and IL- β) and neutrophil infiltration	[182]
		Rolipram	Reduced neutrophil infiltration and inflammatory cytokine production, such as IL- β , TNF- α and TGF- β	[178]
	PARP-1 inhibitor	PJ34	Reduced microglia activation and astrogliosis	[191]

SMIs currently being evaluated for reducing cerebral ischemia in experimental animal models. SMIs in bold are mentioned in the text.

ICAM-1: Intracellular adhesion molecule-1; iNOS: Inducible NOS; LOX: Lipoxigenase; NOS: Nitric oxide synthase; PARP: Poly(ADP-ribose) polymerase; ROS: Reactive oxygen species; SMI: Small molecule inhibitor.

Table 2.

Neuroprotective agents in Phases II, III and IV clinical trials (internet stroke center, 2013).

Pathophysiological target	Classification	Agent	Trials	Phase	Status	Ref.
Thrombosis	Anti-coagulants/anti-platelet	Dabigatran etexilate	Randomized evaluation of long-term anti-coagulant therapy (RELY) with dabigatran etexilate	III	Completed	[30]
		Eptifibatide	Study of the combination therapy of rt-PA and eptifibatide to treat acute ischemic stroke (CLEAR-ER)	II	Completed	[200]
		Rivaroxaban	An efficacy and safety study of rivaroxaban with Warfarin for the prevention of stroke and non-CNS systemic embolism in patients with non-valvular atrial fibrillation	III	Completed	[201]
Ischemic cascade	Ca ²⁺ channel inhibitor	Ximelagatran	Melagatran/ximelagatran versus enoxaparin for the prevention of venous thromboembolic events (EXTEND)	III	Terminated	[202]
		Apixaban	Apixaban for the prevention of stroke in subjects with atrial fibrillation (ARISTOTLE)	III	Completed	[41]
		Magnesium	Field administration of stroke therapy-magnesium (FAST-MAG) trial	III	Ongoing	[203]
		Nimodipine	Efficacy and safety study of Nimodipine to prevent mild cognitive impairment after acute ischemic strokes (NICE)	IV	Ongoing	[81]
Mitochondrial injury	NMDA receptor antagonists	Aptiganel Selfotel	Aptiganel acute stroke trial Acute stroke studies involving Selfotel treatment	III III	Ongoing No benefit	[204] [205]
		GV150526	GAIN 1 and GAIN 2	II	Completed	[63]
		Edaravone	Edaravone-sodium ozagrel comparative post-marketing study on acute ischemic stroke and EAST	IV	Clinically available in Japan	[120]
		Tirilazad	Randomized trial of tirilazad mesylate in acute stroke	III	No benefit	[206]
Inflammation	Phosphatidylcholine precursor	NX-059	Safety and effectiveness of NX-059 for the treatment of patients who have suffered from a stroke	II/III	Failed	[207]
		Citicoline	ICTUS study: international Citicoline trial on acute stroke	III	Terminated	[208]

Current and past clinical trials using small molecule inhibitors (SMIs) as a form of treatment. SMIs in bold are mentioned in the text. GAIN: Glycine antagonist in neuroprotection.