Review Article

Neuroprotectives in Acute Ischemic Stroke, Hope or Hype?

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Abstract

Stroke is a significant public health burden which absolutely requires more effective therapies. The approved treatment options for stroke including tissue plasminogen activators, antiplatelet agents and anticoagulants mainly bear antithrombotic effects. Meanwhile, evolving investigational approaches such as collateral therapeutics and neuroprotective agents has thus far been attempted with equivocal effects on stroke outcome. The basic structural and ultrastructural changes following acute ischemic stroke should be well-considered when trying to target oxidative stress and cell death pathways using neuroprotective agents. Clearly, the positive results of preclinical studies on neuroprotectives and collateral therapeutics in stroke do not necessarily translate to the true clinical benefits of these agents. As such, several large advance-phased trials have already failed to prove so. On the other hand, controversial results in clinical setting should not discourage further research endeavors on the same. Besides, the concurrent use of flow augmentation and neuroprotectives may serve further clinical benefits. Based on the available evidence, it appears that optimization of preclinical studies and further well-designed prospective clinical trials let neuroprotection possibly find its position in stroke management. The present paper discusses key preclinical and clinical studies on neuroprotectives towards improved outcome in acute ischemic stroke.

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Introduction

Stroke as the second most common cause of death and the leading cause of acquired major disability afflicts millions of people worldwide (1-3). Despite the current therapeutic interventions, the outcome of ischemic stroke remains suboptimal if not poor in many instances. Stroke is a formidable disorder with few approved therapies to date. While the ischemic insults mainly at the core of the ischemic zone results in irreversible cell death, novel approaches which root in fundamental neuroscience insights tend to offer hope for a better clinical outcome in ischemic stroke. Consensus and controversies on these approaches continue to emerge. These interventions include but not restricted to collateral cerebral blood flow augmentation (4), neuroprotective treatments (5, 6), stem cell therapy (7) and cortical brain

stimulation (8, 9). The ischemic stress resulting from the occlusion of a cerebral blood vessel triggers neuroinflammation and ultimately results in cell death in acute stroke. Meanwhile, the severity and reversibility of such a damage substantially depend on how preserved is the blood flow and how extensive is the ischemic insult (10).

Cerebral ischemia is shown to initiate a chain of detrimental neuroinflammatory events such as glutamate-mediated excitotoxicity, intracellular calcium accumulation and eventually DNA damage which all negatively affect the cellular homeostasis and lead to structural damage within the ischemic brain tissue (10). Cerebral ischemia causes accumulation of free radicals resulting in damage to nerve tissue in the brain. At synaptic and cellular levels, the role of contributing neurotransmitters, ion channels and pumps' failure have been discussed in the literature (11,12).

The brain is uniquely vulnerable to oxidative damage and so intolerant to blood flow interruptions. Given the circuit-based function of the brain, any small deficit may result in a huge impact. Over the course of acute stroke, major cellular and metabolic perturbations resulting from nitrative and oxidative stress, cessation of mitochondrial activity, lipolysis, proteolysis and microtubular disintegration make the affected tissue succumb through apoptosis (11). Reactive oxygen species (ROS) play a key part in oxidative stress and the ultimate cell fate, which is apoptosis. Multiple sources of ROS generation such as monoamine oxidase (MAO), aconitase, α-ketoglutarate dehydrogenase (a-KGDH), Nox(s), complex I, P450s and neurotrophic factor withdrawal give rise to the oxidative insult following ischemic stress (13, 14). Upon oxidative stress, mitochondrial catalase becomes low, thus the antioxidant and repair capacity would be quite limited (12,13).

On the other hand, while the decreased blood flow (below 20% of the baseline) in the core of stroke and the resultant ischemic stress lead to rapid cell death, the penumbral tissue which surrounds the core has a partially maintained blood flow, hence subject to delayed apoptosis (13).

During the focal ischemic stroke, when a primary vascular route is blocked, compensatory mechanisms come into play. As such, collateral vascular pathways which are auxiliary cerebrovascular anastomoses, primarily maintain partial perfusion of the penumbral ischemic tissue after stroke (15).

Moreover, since the functionality of neural cells and their membranes' ion gradient maintenance depend on the brain's aerobic metabolism, oxygenation, preservation of blood flow and the prevention of neuroinflammatory insults (11), any treatment which either restores collateral blood flow to the penumbral tissue or slows down the neuroinflammatory processes is expected to at least partly reduce neuronal damage and improve the stroke outcome.

Further to the investigational cerebrovascular and neuroprotective approaches to improve outcome in ischemic stroke, rich concepts such as neural plasticity and the remarkable neuronal capacity in synaptogenesis have provided physician-scientists a wide window of hope for neuronal repair following stroke.

Several preclinical investigations such as novel pharmacological intervention using neuromodulators (noradrenergic or cholinergic stimulation) or neural growth factors [including brain derived neurotrophic factor (BDNF), inosine, recombinant erythropoietin and Nogo-A protein], transcranial magnetic- or direct electrical cortical stimulation and cell replacement therapies have offered promising results yet in rodent stroke models (11).

Taken together, there are a dozen of potential therapeutic approaches beyond neuroprotectives (e.g. cell replacement therapy, flow augmentation and cortical stimulation) which may potentially be considered as an additional treatment options for ischemic stroke in few decades (7). Meanwhile, in this brief review, we have focused on appraising neuroprotective preclinical and clinical findings in the literature. Besides, the questions of "whether and how the combination of flow augmentation (improved collateral circulation) and administration of neuroprotective agents serve synergistic protective effects and how such investigational protocols could even possibly improve outcome in human stroke" are addressed.

Collateral blood flow in ischemic stroke

The term 'collateral therapeutics' encompasses the treatment measures which improve penumbral tissue perfusion upon acute ischemic stroke (15). The improved perfusion from a patent artery into the territory of an obstructed one, is primarily resulted from the harnessed vascular redundancies and tiny endogenous anastomoses between the distal branches of the cerebral arteries which potentially reduce the extent of the ischemic insult (13, 15). Moreover, augmented collaterals (leptomeningeal or pial) form these anastomotic links may facilitate the delivery of thrombolytic and other neuroprotective agents and potentiate the efficacy of the selected therapeutic strategy on the affected tissue (16, 17).

Despite significant variations in leptomeningeal collateral configurations, stroke patients with better collateral scores (evaluated during angiography) tend to have less infarct volume and more favorable clinical

outcome measures (for instance less Rankin scale scores) upon discharge. Furthermore, better collateral grade is known to diminish the risk for hemorrhagic transformation and helps better recanalization following endovascular treatments (17,18).

To augment the cerebral collateral blood flow, several techniques have been proposed with their efficacy and safety investigated. These methods include mild induced hypertension, intravascular volume expansion, vasodilation, and sphenopalatine ganglion stimulation. The preclinical and clinical investigation of the above approaches has variably demonstrated collateral augmentation with increased blood flow and resultant neuroprotective effects. Nevertheless, many of these strategies remain under-developed and their effects on enhancing collateral blood flow during stroke are yet to be confirmed (14, 18).

With regards to induced-hypertension as a strategy to augment collaterals, some clinical evidence has suggested enhanced cerebral perfusion, decreased hypoperfused tissue volume and improved NIH Stroke Scale (NIHSS) with only minimal increase in intracranial pressure, which make this an alternative strategy especially in patients not eligible to receive thrombolysis. Even though, the safety of such an approach is yet undefined as applying systemic hypertensive treatments during the course of ischemic stroke may contribute to further risk for intracerebral hemorrhage, reflex bradycardia, and ischemic bowel disease (19).

The inhaled nitric oxide-induced vasodilation has also introduced some beneficial preclinical effects in traumatic brain injury and ischemic stroke (20). Animal studies have substantiated that inhaled nitric oxide in a middle cerebral artery occlusion (MCAo) stroke model, selectively dilated the ischemic penumbra arterioles; hence augmented the collateral circulation and diminished the ischemia-induced damage (18, 20, 21).

Along these lines, some other preclinical findings have supported the beneficial effects of the electrical stimulation of sphenopalatine ganglion which projects parasympathetic outputs towards anterior cerebral circulation(14). The above approach is also shown to enhance ipsilateral perfusion and improve outcome in stroke animal models, yet the direct extrapolation of such benefits to stroke recovery in human seems not to be possible at this stage (22). Further studies on this subject are ongoing.

Some recent evidence on collateral therapeutics have emerged from preclinical and clinical investigations on a method known as transient aortic occlusion (TAO) (13). According to recent rodent studies, TAO has led to a reduced infarct zone size, 24 hours after the thromboembolic MCAo (13). At clinical level, the landmark SENTIS trials (4,13,23-26) have demonstrated reasonable safety profile for TAO and demonstrated that such an approach may improve stroke outcome in distinct group of patients. Moreover, evaluating the effectiveness of TAO in a non-stroke porcine model has suggested an enhanced cerebral blood flow at least by 30% (27). However, there is no available mechanistic data explaining the effect of TAO on augmented leptomeningeal collateral circulation in the brain (13).

Research on 'collateral therapeutics' and the influence of various collateral augmenting interventions on ischemic stroke outcome is emerging.

The role of neuroprotection in acute ischemic stroke outcome

The neuroimflammatory processes triggered in stroke contribute to the activation of intra-cytosolic apoptotic pathways in neural cells. As such, the proapoptotic factors such as mitochondrial Cyt-C, BCL2, Bax, and caspase-3 regulate the process of cell death (12).

Neuroprotective biomarkers such as the brain derived neurotrophic factor (BDNF) (28) and neural growth factor-1 (NGF-1) (29) has been shown to counteract the cell death process in the face of oxidative or nitrative stress. Moreover, the level of antioxidative enzymes including superoxide dismutase (SOD), Glutathione (GSH) and catalase were shown to be inversely proportional to the oxidative damage resulting from the neuroinflammatory processes during acute ischemic stroke (11, 30, 31).

On the other hand, the rate of ischemic neuronal cell death has been suggested to, at least partly, depend on intracellular glucocorticoid levels which are mediated by the enzyme called 11beta-hydroxysteroid dehydrogenase type 1 (11β-HSD1)(6). Therefore, inhibition of this enzyme may be hold potential therapeutic value when neuroprotective therapies are administered in ischemic stroke (6). The role of proinflammatory mediators such as Interleukins (IL-1 and IL-6, for instance) (32) and tumor necrosis factor alpha (TNF-α) (33) upon acute stress-related neuroinflammation and apoptotic processes in the brain have been well discussed in the literature (31-33).

Many studies which tried to assess the effectiveness of neuroprotective agents in acute ischemic stroke have employed MCAo method as an ischemia/reperfusion (I/R) model in rodents.

There are several agents with their established cerebroprotective effects described in preclinical and clinical experiments (7, 14, 29, 30, 34-36). For instance, studies which evaluated the therapeutic effects of Coccomyxa gloeobotrydiformis (CGD), a genus of algae, in the family Coccomyxaceae, on ischemic stroke in a rat model of MCAo, measured the infarct volumes, neurologic deficits and degree of stroke-induced brain edema in CGD-treated animals 24 hours after reperfusion. Recent findings have substantiated some CGD's beneficial effect through inducing mitochondrial protection and anti-apoptotic mechanisms (35,36).

In addition, chronic administration of agents with plausible neuroprotective effects such as chloroform and petroleum ether extracts of Nigella sativa seeds to MCAo stroke model of rat has led to infarct volume reduction as well as expression of antioxidative enzymes including GSH, SOD and catalase (34).

Furthermore, a recent experiment on the effectiveness of parthenolide in a rat model of ischemic stroke demonstrated neuroprotective effects of this agent against the damage induced by MCAo. Such effects were suggested to depend on the down-regulation of NF- kappa B (nuclear factor kappa-light-chain-enhancer of activated B cells), phospho-p38 MAPK, and caspase-1 expressions and amelioration of the blood-brain barrier permeability (37). In a similar study, administration of bicyclol to rat model of ischemic stroke demonstrated protective effects on cerebral ischemia, and such protection was attributed to the down-regulating of toll-like receptors 4 and 9 (TLR4 and TLR9), TNF receptor associated factor (TRAF6), NF-kappaB, matrix metallopeptidase 9 (MMP-9) and up-regulation of claudin-5 expression (38).

Flavonoids are generally reported to possess anti-inflammatory properties (39), thus their antioxidative and neuroprotective effects have been investigated in several studies. Based on the findings of a recent work, when MCAo rats were treated with kaempferol in a postischemia setting, they showed less ischemic brain injury (39). The attenuated neuroinflammatory response through the inhibition of the signal transducer and activator of transcription-3 (STAT3) and NF-kappaB activation may partly explain the potential mechanism(s) involved. Likewise, the effects of another flavonoid called naringenin on the outcome of acute ischemic stroke was examined. Findings revealed that prophylactic treatment with naringenin may improve the functional outcomes and abrogate the ischemic brain insult (39).

Similarly, some recent preclinical and clinical investigations have evaluated the effect of potentially neuroprotective agents in the outcome of acute stroke. Results of the studies which assess such effects following administration of carnosine (35), insulin-like growth factor-1(29), nitric oxide donors (40) and normobaric hyperoxia-based neuroprotective therapies (41) are intriguing.

Cerebrolysin is a medicinal product from neuropeptide with low molecular weight and free aminoacids. It has been shown to exert considerable effects on excitotoxicity, inhibition of free radicals formation, activation of microglia and neurotrophic activity, creation of neuron sprouts, improvement of cell life and neurogenetic degeneration following brain attack (42). Based on some in vivo results, cerebrolysin potentially improved neurological outcome and neurogenesis in ischemic brain attack in rodents (43). Cerebrolysin in tissue culture models of neuronal ischemia and related clinical studies was shown not only to increase neuronal survival but also to improve outcome from hemorrhagic and spinal injury. Improvement in learning and memory as well as reduced anxiety were reported in cerebrolysin consumers (44). Some studies have demonstrated that cerebrolysin is effective in neuronal development and brain metabolism. Wenzel et al. in 1981 corroborated that cerebrolysin increases dendritic spines in the hippocampi of neonatal rats. Later studies documented that cerebrolysin increases glutamate transporter (GLUT-1) expression in blood brain barrier. As a result, it can prevent blood brain barrier damage following brain attack. Cerebrolysin in different models of brain ischemia has reduced apoptosis by diminishing hydroxyl free radicals in the brain and also decreased cerebral infract after focal ischemia in rat. This can be considered as the possible mechanism through which cerebrolysin provides neuroprotective effects (43, 44).

NeuroAiD (MLC601/MLC901) is a traditional Chinese medicine (TCM) comprising 9 plants and 5 animal's parts with well-supported neurological and cellular functions in brain-attack animal models (45). The neural repair properties of this medicine have been reported in several animal models. This agent was shown to be especially effective when administered during the early hours after brain attack. NeuroAiD may promote neurogenesis, improve inter-neuronal links and form dendrites and new synapses after brain ischemic insult (46). Evolving evidence have supported its role in proliferation and differentiation of new neural cells as well as neural repair following stroke (47, 48). Nevertheless, further research are required to establish its true clinical benefit in randomized and controlled clinical settings.

The role of free radicals and calcium-mediated excitotoxicity in ischemic insults following acute stroke is well documented. It has been proposed that anti-inflammatory and anti-oxidant agents may alleviate ischemic cerebral injury. In a recent investigation, our study-group examined the possible beneficial effects of an antioxidant (canola oil from rapeseed plants Brasica napus) on neurological and behavioral outcomes after cerebral ischemia. The neuroprotective effect of dietary canola oil against middle cerebral artery occlusion (MCAo)-induced cerebral ischemia injury was evaluated in rats. Results indicated motor performance improvement in animals treated with canola oil as compared to the control group. Such

findings suggest that administration of canola oil may exert protective effects in an experimental rat model of cerebral ischemia (unpublished data).

In another of our studies (49), we examined the effects of immunomodulatory drug Setarud, a mixture of herbal extracts (Rosa canina, Urtica dioica, Tanacetum vulgare) supplemented with selenium, on cerebral ischemia in male rats. Rats were interaperitoneally injected with 0.66 mL/kg Setarud for 30 minutes after MCAo. Based on triphenyltetrazolium chloride staining, Setarud was found to reduce the cerebral infarct volume of rats subjected to cerebral ischemia. Additionally, behavioral tests showed that Setarud could significantly improve the motor function of rats with cerebral ischemia. Based on these findings, Setarud demonstrated neuroprotective effects against ischemic brain injury.

Neuroprotective treatments have so far been evaluated in considerable number of studies to see whether they can improve the outcome of acute ischemic stroke. However, results from clinical studies have not been encouraging. For instance, the outcome of SAINT I trial (assessing the effects of NXY-059 on stroke outcome) was marginally positive while the SAINT II Trial, a large randomized multicenter clinical trial of the NXY-059, failed to demonstrate a treatment benefit in acute ischemic stroke halting further clinical development (24). On the other hand, a notable set of clinical data on the efficacy and safety of TAO as a collateral augmenting approach in ischemic stroke (from SENTIS trial) has evaluated such a method in a large clinical cohort of ischemic stroke (4, 23-26). Nonetheless, clinical application of the cerebral perfusion augmentation via transient and partial aortic occlusion in acute ischemic stroke is yet to be further justified.

With regard to neuroprotective treatments, animal models of stroke have been used and the cellular-molecular impact of such therapies have been examined in ex-vivo brain homogenates of rodents' brain subjected to MCAo. To date, well-controlled clinical studies are lacking due to limitations in response assessment methods. The histopathological and cellular-molecular assays on brain tissue is yet difficult to be brought into clinical setting except in the scope of autopsy-obtained tissues. It is also hard to extrapolate serum concentrations on neurotrofic factors to their levels in specific parts of the brain. However some circulating factors including BDNF are known to represent their concentration in distinct brain regions (50).

Conclusive remarks

So far, some preclinical and few clinical studies on neuroprotective use in stoke have shown promising results in early phases. Meanwhile much of these efforts have failed to prove any solid evidence for clinical efficacy in large scale.

Some major factors including: 1- the physiochemical properties of the studied neuroprotectives, 2- their polar, nonlipophilic nature with poor blood–brain barrier penetrability, 3-non-physiological oxidation potential and low potency, 4-lack of biomarkers assessing oxidative stress and 5-the heterogeneity in individual human responses to drug treatment seem to be the main drawbacks in most clinical studies on neuroprotectives and stroke. Other than clinical research, preclinical studies also need more optimization.

Given the above, despite the promising results showing that several neuroprotective agents such as flavonoids can hamper ischemic damages following stroke in animal models, such results are still far from direct extrapolation to stroke outcome in human and there seem to be missing steps for jumping into stroke clinical trials through these approaches.

The available evidence on the effects of neuroprotectives in stroke should not be overvalued as long-term data are lacking. Nevertheless, the so far equivocal results in collateral therapeutics'- and neuroprotectives' effects on stroke outcome should not discourage further well-planned research endeavors.

Future perspectives

There exists some key questions before promoting neuroprotective treatments in stroke. For instance, the position of anti-inflammatory agents in the management of stroke, the possible synergy of administered neuroprotective agents together with collateral augmentation methods such as TAO, the possible benefit of using tissue plasminogen activator (tPA) together with collateral augmentation or neuroprotective therapies for the management of stroke, are among unresolved issues. Well-designed basic, translational and clinical neuroscience studies are needed to elaborate on the above.

To reach better positions in ischemic stroke management, research on neuroprotection, vascular interventions, cell replacement therapies, novel pharmacological approaches and cortical brain stimulation need to gain momentum.

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