Review

The future of neuroprotection in stroke

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ABSTRACT

Investigators acknowledge the limitations of rodent or non-human primate stroke models, hundreds of putative neuroprotectants have been evaluated in preclinical models, but not one has entered the clinical realm. Initial studies focused on the neuron, but in recent years the focus has widened to also include other neural cells including astrocytes, pericytes and endothelial cells, which together form the neurovascular unit. Some new developments raise renewed hope for neuroprotection: the appearance of new compounds with multiple mechanisms of action, or the promulgation of new standards for a rigorous preclinical testing. At the bedside in the last 5 years, uric acid and nerinetide are the only compounds tested for clinical efficacy in randomised controlled trials (RCTs), where all patients had to receive reperfusion therapies, either intravenous thrombolysis and/or mechanical thrombectomy. In addition, otaplimastat, 3K3A-activated protein C (APC), intra-arterial verapamil and intra-arterial hypothermia were also assessed in combination with reperfusion therapy, but in RCTs that only included feasibility or safety outcomes. Some of these compounds yielded promising results which are discussed in this review. Altogether, a deeper knowledge of the mechanisms involved in the ischaemic death process at the neurovascular unit, an improved preselection and evaluation of drugs at the preclinical stage and the testing of putative neuroprotectants in enriched clinical studies of patients receiving reperfusion therapies, might prove more effective than in the past to reverse a dismal situation that has lasted already too long.

INTRODUCTION

The therapy of acute ischaemic stroke (AIS) has evolved in the last 5 years with the recognition of the value of thrombectomy in properly selected patients, or the utility of brain imaging techniques to individualise the use of thrombectomy up to 24 hours after stroke onset including wake-up strokes.[1 2] Nonetheless, stroke remains a significant burden on patients, families and the global healthcare system, for even the benefits of reperfusion therapies are incomplete in about half of treated patients. In addition to recanalisation, treatment with compounds designed to protect brain cells during ischaemia seems a reasonable treatment alternative.[3] Over the past quarter century, quite literally hundreds of putative neuroprotectants have been evaluated in preclinical models, but not one has entered the clinical realm.[4] The specific problems that may account for prior translational failures have been well enumerated by others[5 6] (table 1). Interest in brain protection has been rekindled by three major events. First, the development of endovascular thrombectomy (EVT) as a viable treatment option galvanised the stroke community. Not just a new, effective treatment for patients, EVT offers the opportunity to examine—or in some cases re-examine—brain protective therapies in combination with recanalisation. Looking back at prior clinical trial failures, one notes a profound lack of attention to the recanalisation status of patients. One lesson from animal studies that likely applies to human patients with stroke is that brain protective therapies are much more likely to succeed when used in reversible occlusion models, that is, the animal version of EVT. Second, newer agents have been developed that harbour multiple mechanisms of action. For example, uric acid (UA), NA-1 and 3K3A-APC act on multiple targets in the nervous system and induce multiple cytoprotective effects. Prior failures involved drugs that acted only on one putative important pathway or receptor in the ischaemic cascade. Third, a deeper understanding of rigour in the laboratory has led to new guidelines, for example, RIGOR, CAMAREDES and ARRIVE. For all three of these reasons, we sought to describe the potential for future clinical trial success. This review non-systematically updates the main advances produced in the field according to the opinion of the authors who have been fully involved in them during the last decades.

Neuroprotection: new developments at the bench

Neuroprotection in ischaemic stroke is an evolving concept generally geared towards preserving brain matter that is compromised by ischaemia and, if left untreated, will go on to die. It is closely linked to the penumbra, the tissue which is not directly damaged by the stroke but in which hypoperfusion and metabolic changes occur that put the tissue at risk of delayed cell death. While cells in the infarct core are seen as anoxic and irretrievably lost, the surrounding hypoperfused penumbra region features tissue that initially is viable but at risk of delayed cell death. This is the territory that neuroprotection aims to preserve. Initial studies focused on the neuron, but in recent years the focus has widened to also include other neural cells including astrocytes, pericytes and endothelial cells, which together form the neurovascular unit. We here cover different aspects of neuroprotection in the categories cell death, reperfusion injury and events around the neurovascular unit.

Cell death

A number of pathways towards cell death are activated in experimental stroke, covering the spectrum from apoptosis to necrosis and involving different

Table 1 Reasons for failure to translate preclinical promise to clinical trial success

Preclinical		Clinical	
Problem	**Solution**	**Problem**	**Solution**
Selection bias	Randomisation	Power	Large phase III trials
Assessment bias	Blind outcome assessment	Time window	Require early enrolment
Power	Prespecified sample size	Recanalisation	Require documented recanalisation
Reproducibility	Repeat in multiple labs		
Attrition bias	Subject tracking	Heterogeneity	Broad inclusion criteria
Generalisability	Repeat in more than one model		
Relevance and external validity	Evaluate in animals with comorbidities of both sexes and after ageing		

cell types. Apoptotic mechanisms can include caspase activation, but at least in experimental stroke appear to be mediated more via mitochondrial damage and translocation of apoptosis-inducing factor to the cell nucleus. At the other end of the spectrum, a role for ferroptosis/oxytosis as well as for necroptosis has been postulated.[7] The initial focus of neuroprotective efforts concerned mostly the rescue of neurons, seen as the cells most vulnerable to both oxygen deprivation,[8] as well as the excitotoxicity induced by excessive stimulation of neuronal glutamate receptors. As a consequence of excitotoxicity and related events, the normally carefully calibrated ion balance of neural cells is disrupted, and ion channels have thus become targets for neuroprotection. Another important early event is the loss of glutathione, the major antioxidant defence of neurons. The resulting oxidative stress is a major factor in neuronal cell death, and thus early neuroprotective strategies often focused on the use of antioxidants and radical scavengers. Despite infarct size reductions in animal models these have generally not translated well to stroke treatment in humans, perhaps due to a combination of low specificity and lack of access to the cells where they were needed most. This realisation has in recent years shifted the preclinical focus more towards ramping up the expression of protective antioxidant enzymes,[9] as well as targeting the downstream effector enzymes that are activated by and contribute to propagating oxidative stress such as lipoxygenases, cyclooxygenase and cytochrome P450.[10] As in all neuroprotective strategies, timing is critical.

Reperfusion injury

When blood flow is restored early after onset of ischaemia, it is beneficial and can greatly reduce the infarct. However, when reperfusion occurs late a seemingly paradoxical increased injury can occur. This reperfusion injury may be caused by several factors, including the ischaemia-induced upregulation of a number of redox enzymes that are now presented with an abundance of substrate oxygen. This in turn leads to lipid peroxidation and organelle damage, resulting in increased cell death and a greater infarct. These mechanisms may also work in concert with the physicochemical stress of oxygen-rich blood suddenly hitting a vasculature weakened by the ischaemia. This latter factor which is aptly modelled in the filament model of transient middle cerebral artery occlusion (tMCAO).[11] Another important aspect of reperfusion injury is the possibility of secondary bleeding, that is, haemorrhagic transformation (HT). Limiting HT subsequent to tPA thrombolysis is a major neuroprotective goal, as this is a major reason why tPA is given to such a limited percentage of patients with stroke. This is particularly relevant for patients on oral anticoagulants or antiplatelet medication, and there are mouse models for both of these scenarios.[12 13] In a mouse model with warfarin-treated mice, lipoxygenase inhibition provided neuroprotection in terms of reduced infarct size, and greatly reduced HT.[14] Additional and inter-related factors are autophagy, the regulation of which is increasingly recognised as possible drug target,[14] and inflammatory mechanisms, which depending on time and location can be both injurious, and protective.

Neurovascular unit

The idea of the neurovascular unit has provided a fruitful conceptual framework for investigating mechanisms of stroke pathophysiology. We survey three areas of recent advance and refinement of this overall concept: (i) help-me signalling mediates injury-into-recovery transition within the neurovascular unit during stroke recovery; (ii) the neurovascular unit communicates with systemic physiology and (iii) the neurovascular unit is affected by systemic variables such as age, comorbidities and circadian rhythm.

The penumbra provides hope and opportunity in stroke because cells in the penumbra do not immediately die after ischaemic onset. Instead, the penumbra succumbs over time, depending on the degree of intermediate perfusion that is sustained by collaterals. In recent years, it is now recognised that the penumbra is not just actively dying; it is also actively trying to recover, and many mediators play biphasic roles—deleterious in the acute stage but potentially beneficial during recovery.[15] For example, the alarmin HMGB1 is neurotoxic during acute stroke but may be released by reactive astrocytes in the delayed phase as a homing signal for endothelial progenitors in order to amplify angiogenesis. The overall concept of help-me signalling has been proposed as a framework to dissect these endogenous recovery pathways in the injured central nervous system.[16] Damaged but not yet dead cells can recruit the exchange of help-me signals within the remodelling neurovascular unit, including lipocalin-2,[17] vascular endothelial growth factor[18] and even extracellular mitochondria.[19]

Another recent modification of the neurovascular unit concept involves the recognition that the unit is not isolated within the cranium. Instead, the brain possesses a glymphatic drainage, and function and dysfunction in this system mediates neurodegeneration.[20] It is likely that analogous pathways operate in stroke pathophysiology. For example, glymphatic mechanisms contribute to oedema after focal ischaemia,[21] and glymphatic drainage from brain-to-cervical lymph nodes triggers peripheral inflammation and secondary brain damage after stroke.[22] Another recent study suggested that circulating monocytes replace perivascular macrophages after stroke,[23] thus demonstrating that systemic responses may directly remodel the neurovascular unit as well.

Because the neurovascular unit communicates with the peripheral system, it is likely that systemic physiology influences how the neurovascular unit responds to stroke or potential therapies.

Transcriptome mapping of the gliome and vasculome reveals that these components of the neurovascular unit are affected by age, hypertension and diabetes,[24 25] and the diseased vasculome may be partly renormalised by positive modulation such as exercise.[26] A comorbid neurovascular unit is more difficult to protect. For example, normobaric hyperoxia reduced infarction in normotensive rats but not hypertensive rats.[27] Finally, a recent study hypothesised that systemic circadian rhythms may also affect how the neurovascular unit responds to therapy after stroke.[28] All these emerging findings highlight the potential importance of including age, sex, comorbidities and circadian parameters in preclinical drug testing paradigms for stroke. Including additional variables adds to cost and duration of the preclinical testing phase, and this would need to be acknowledged by funding institutions like the National Institutes of Health, as well as by journals dedicated to publishing translational studies. Accommodating and even rewarding these types of 'semi-replication' studies would go a long way to creating more robust results that may better translate to the treatment of human stroke.

Improving the efficiency of translational research in stroke

Preclinical stroke models seek to recapitulate a few aspects of human stroke for the purpose of screening candidate protectant treatments for benefit. Investigators acknowledge the limitations of rodent or non-human primate stroke models, but believe that before beginning large, expensive clinical trials it is important to demonstrate some evidence of improved outcome in an animal stroke model. As listed in table 1, prior work has often lacked adherence to full scientific rigour and best practice. Randomisation is essential to reducing selection bias. Outcomes—behavioural ratings, histological measurements—have not always been performed in a blinded fashion, leading to possible bias from investigators leaning toward one outcome or another. Underpowered experiments can lead investigators to publish as soon as they achieve a statistically significant result; this can be overcome with a prespecified treatment effect size and sample size. Reproducibility and generalisability refer to the observation that some results cannot be replicated outside of the laboratory that generated the initial positive result. Many authorities therefore recommend requiring positive results from several labs using more than one type of model. Attrition bias refers to the common habit in basic science laboratories of ignoring 'drop-out' animals and replacing them with additional subjects. The 'drop-outs' could reflect biological realities that could influence outcome in the stroke model and is overcome by including all subjects enrolled into the experiment, even if they 'drop-out' prior to the final outcome. Publication bias, the tendency to publish only positive results, can be overcome by trial registration or posting the experimental protocol on a pre-print server.

Clinical trial failures include some issues in common with preclinical failures, but many were rectified long ago. For example, ascertainment bias does not occur in clinical trials if investigators are truly blinded to which treatment group the patient received. Time window problems refer to the observation that many stroke treatments work well in animals when delivered shortly after the onset of the cerebral ischaemia, yet in clinical trials, subject enrolment is allowed out to a long time after stroke onset because early recruitment is deemed too difficult. Power analysis, treatment effects and sample sizes are routinely specified prior to the clinical trial beginning. Heterogeneity among subjects is a complicated topic with significant issues on both sides. On the one hand, the more homogeneous the population the easier it might be to demonstrate a treatment effect, by avoiding enrolment of non-informative patients. On the other hand, stroke is a heterogenous condition and if clinical trials reflect this, the results should be more generalisable.

In the past 5 years, two significant developments raise new hope for neuroprotection: the appearance of new compounds with multiple mechanisms of action, and the promulgation of new standards for the rigorous preclinical development beyond the venerable Stroke Treatment Academic Industry Roundtable (STAIR) guidelines. Established dogma dictated that putative neuroprotectants must have one firmly established mechanism of action, typically involving a single target. Multifunctional treatments, for example, therapeutic hypothermia, attracted little interest because the single mechanism of action could not be identified. Progressively, our field accepted the complexity of the ischaemic cascade: that multiple deleterious pathways proceeded in parallel and that successful neuroprotectants should target multiple pathways. To repeat, however, in the absence of any clinical success, ideas about the best way to select the ideal neuroprotectants remain speculative.

The second significant development raising new hope for neuroprotection concerns the evolution of testing guidelines after STAIR. Intense analysis of preclinical development programmes in stroke and neurodegeneration have identified key problems that must be addressed, starting with a variety of biases that have bedevilled animal research in general, but stroke modelling specifically: attrition bias, detection bias, performance bias and selection bias. Working groups in Europe and in the USA identified the need to address these problems in a coordinated manner. The consensus includes an appreciation that multisite preclinical trials are needed. Such multicentre trials must include key design elements that will overcome prior failures.[29 30]

THE SPAN PROJECT

In 2018, the US National Institutes of Neurological Disorders and Stroke (NINDS) requested applications for participants in the Stroke Preclinical Assessment Network (SPAN). Through competitive peer review, NINDS selected six study sites and one coordinating centre. SPAN is intended to achieve significant improvement and advancement of preclinical development by implementing the following critical technical innovations for the first time in a preclinical stroke testing network: central randomisation, masking treatment assignment, power analysis and rational sample sizing, replication in multiple laboratories, study with key factors that impact outcome, for example, diabetes, hypertension, age, sex. In SPAN, preclinical investigators will use, for the first time, essential elements of rigour typically found in clinical trials. For example, SPAN will require registration of all subjects on arrival at the study site, using a permanent bar-coded ear tag. Thus, if subjects drop out prior to randomisation, they will be accounted for. Randomisation will occur via a centralised scheme, to avoid local investigator bias. Study compounds have been packaged in identical appearing, masked vials to be shipped to sites from a central pharmacy. To blind the behavioural assessments, local investigators will record and upload video of the subject performing the behavioural task. Then, in a masked fashion, the video will be viewed and scored by other investigators unaware of the treatment provided, the site where the video was recorded, or the time sequence of the recording (prestroke or poststroke). In addition to the above innovations, simulations have clearly documented the superiority of multisite trials over larger single-laboratory studies. The multisite approach improves the external validity and should

improve the likelihood of clinical success. Each of the putative neuroprotectants will be tested at all six study sites. Heterogeneity among the study sites is expected, so positive results should have far greater generalisability.

Neuroprotection and reperfusion therapy at the bedside

Recently completed stroke trials

A non-systematic search in PubMed restricting the selection criteria to studies in patients treated with reperfusion therapy and conducted in the last 5 years yielded UA and nerinetide as the only compounds assessed in efficacy trials and otaplimastat, 3K3A-APC, intra-arterial verapamil and intra-arterial hypothermia, as therapies assessed in feasibility or safety trials.

Uric acid

UA is the final product of purine catabolism in humans and acts as a potent endogenous antioxidant that scavenges reactive nitrogen and oxygen radicals and non-radicals (such as peroxynitrite), exerting strong and multifaceted neuroprotective effects in preclinical stroke models.[31] The generation of UA is increased in the ischaemic brain, but there is a gradual exhaustion of the antioxidant capacity that correlates with larger infarct volume and worse stroke outcomes. UA is the first neuroprotectant assessed in a RCT where all subjects had to receive alteplase (n=411), and some (n=45), rescue thrombectomy.[32] Overall, 39% of patients in the UA group and 33% in the placebo group obtained an excellent outcome (adjusted risk ratio (RR) 1.23, 95% CI 0.96 to 1.56, p=0.099). Early ischaemic worsening occurred more frequently in the placebo group (9% vs 3% (p=0.01)), and there was a significant interaction between treatment and sex (p=0.045), and treatment and glucose (p=0.016). Indeed, UA therapy in women doubled the effect of placebo to attain the primary outcome (OR 2.08, 95% CI 1.05 to 4.15),[33] and tripled the rate of the primary outcome compared with placebo in patients with hyperglycaemic at stroke onset (OR 2.9, 95% CI 1.0 to 8.3).[34] In patients who received rescue thrombectomy, there was a 19% absolute increment in the rate of good outcome (modified Rankin Scale (mRS) 0 to 2 at day 90) in the UA group compared with placebo (adjusted OR 6.12, 95% CI 1.08 to 34.56).[35] Presumably, increased abundance of substrate oxygen and glucose after thrombectomy and hyperglycaemic, respectively, explained a stronger pro-oxidant drive and the larger treatment effects of UA over placebo in these preplanned enriched analyses. A confirmatory trial of the benefits of UA therapy is planned.

Nerinetide

Nerinetide perturbs postsynaptic density protein 95 protein-protein interactions that lead to excitotoxic cell death in acute ischaemia and reduces stroke damage in animal models of ischaemia/reperfusion including non-human primates. In ESCAPE-NA-1 (Efficacy and safety of nerinetide for the treatment of acute ischaemic stroke),[36] 1105 patients that received thrombectomy were randomly assigned to receive nerinetide or placebo; 61% of patients with nerinetide and 59% with placebo achieved an mRS score of 0–2 at 90 days (adjusted RR 1.04, 95% CI 0.96 to 1.14, p=0.35). Secondary outcomes were also similar between groups and evidenced a treatment effect inhibition in patients also receiving alteplase. Exploratory analyses not adjusted for multiplicity showed an improvement in clinical outcomes, reduction in mortality and reduction in infarct volumes observed in the no-alteplase stratum that received nerinetide, although these findings require confirmation. FRONTIER (Field randomization of NA-1 therapy in early responders, NCT02315443) currently investigates the safety and efficacy of prehospital intravenous NA-1 in the field for AIS within 3 hours of symptom onset, while a new trial is planned to explore the value of nerinetide in patients not receiving thrombolysis before thrombectomy.

Otaplimastat

Otaplimastat inhibits matrix metalloprotease pathway, and reduces oedema and intracerebral haemorrhage induced by alteplase in animal stroke models. In the SAFE-TPA (safety and efficacy of Otaplimastat in patients with acute ischemic stroke requiring tPA) sstrial,[37] 80 patients received intravenous otaplimastat (40 or 80 mg) or placebo after starting the rtPA infusion. The incidence of parenchymal haemorrhage was 0% in the placebo group, 0% in the low-dose of otaplimastat and 4.7% in the high dose of otaplimastat. Low-dose otaplimastat was associated with the greatest proportion of good outcome (mRS at day 90 80% vs 76% placebo). Despite that it was associated with the lowest rate of successful reperfusion (53% vs 74% placebo).

Activated protein C

APC is a blood protease with anticoagulant and cell-signalling activities mediated via the protease-activated receptor 1 (PAR1). APC and analogues with cell-signalling cytoprotective activities provided beneficial effects in preclinical models of stroke. RHAPSODY (safety evaluation of 3K3A-APC in ischemic stroke) was a randomised, controlled, blinded dose-escalation safety trial that treated 110 patients with one of four doses of the pleiotropic PAR1 agonist, 3K3A-APC or placebo after intravenous alteplase, thrombectomy or both. An exploratory analysis found a trend towards lower haemorrhage rate in 3K3A-APC-treated patients, although the incidence of favourable outcome (90 day mRS 0 or 1) was 45% in the treatment group and 63% in the placebo.[38]

Intra-arterial verapamil

Verapamil is a calcium channel blocker used to treat vasospasm. Some reports suggest that verapamil could alter brain glucose content and interact with P-glycoprotein-mediated transportation, which may contribute to blood brain barrier disruption. In SAVER-I (superselective administration of verapamil during recanalization in acute ischemic stroke, NCT02235558), the administration of intra-arterial verapamil in 11 patients with a final TICI 2a to TICI 3 after thrombectomy did not increase the risk of intracranial haemorrhage or other adverse effect/procedural complications. However, the study lacked a control group and the pending important question is whether verapamil adds clinical value following thrombectomy.

Selective intra-arterial hypothermia

Systemic hypothermia had no apparent clinical benefits and increased the risk of pneumonia following AIS in previous studies.[39] Mathematical models estimate that regional hypothermia by a 10 min intra-arterial infusion of cold isotonic saline results in a comparable degree of cooling to systemic hypothermia but with a much faster time to reach hypothermia. Patients with large vessel occlusion within 8 hours after symptom onset were enrolled in a non-randomised, single-arm study of 26 patients that received thrombectomy combined with regional hypothermia. During infusion, rectal temperature decreased 0.1°C, but returned to normal within 5 min after infusion. Selective intra-arterial hypothermia combined with thrombectomy was feasible and safe.[40] However, the study was small,

Table 2 Ongoing neuroprotection trials in patients treated with thrombolysis or thrombectomy

Trial name	Registration #	Intervention	Outcome	Treatment window	Sample size	Estimated completion
REVISE-1	NCT03210051	Remote ischaemic conditioning	Safety Feasibility	6 hours	20	2020
DIAGLUICTUS2	NCT04297345	Peritoneal dialysis	Feasibility Safety	12 hours	20	2021
TESSERACT-BA	NCT0406157	Transcranial electrical stimulation	Safety	Pre-thrombectomy/Post-thrombectomy	24	2022
Verapamil for Neuroprotection in Stroke	NCT03347786	IA verapamil	Safety	8 hours	20	2020
MAVARIC	NCT02912663	Verapamil and magnesium sulfate vs placebo	Safety	Post-thrombectomy	30	2020
SONIC	NCT02831088	Neu2000KWL vs placebo	mRS, safety	8 hours	210	2020
MATRISS	NCT04083001	OTR4132 vs control	Safety	6 hours	18	2021
Imatinib in Acute Ischaemic Stroke	NCT03639922	Imatinib vs placebo	mRS	8 hours	1260	2022
BAST	NCT03539445	Butylphthalide vs placebo	mRS	6 hours	1200	2022
NA	NCT03394950		mRS	4.5 hours	980	2020
AIS	NCT02905565		Safety	24 hours	400	2021
TEXAIS	NCT03287076	Exenatide injection vs control	NIHSS	9 hours	520	2020
SE-GRACE	NCT03284463	Glibenclamide vs placebo	mRS	10 hours	306	2020
CHARM	NCT02864953		mRS	10 hours	680	2021
NA	NCT03062397	JPI-289 vs placebo	Infarct growth	6.5 hours	110	2021

', ' ; NIHSS, National Institutes of Health Stroke Scale.

non-randomised, single arm and observational. Currently, it is not clear whether selective cooling overcomes the widespread objections to the use systemic hypothermia after AIS.

Neuroprotection in stroke: ongoing trials

Several ongoing RCTs are assessing different neuroprotectant strategies in patients receiving concomitantly reperfusion therapies (table 2). Remote ischaemic conditioning (RIC) may remotely trigger self-protective pathways in the brain although through poorly understood mechanisms.[41] RIC administration was found associated with protective effects in prehospital settings, in patients with symptomatic intracranial atherosclerosis and in patients treated with intravenous thrombolysis.[42] The REVISE-1 (remote ischemic conditioning paired with endovascular treatment for acute ischemic stroke) trial is investigating whether RIC is safe and effective in patients with large vessel occlusion undergoing thrombectomy. RIC is performed by an electric auto-control device with cuffs placed on bilateral arms and inflated and deflated 5 times before the endovascular procedure.

Glutamate overload exerts toxic effects on the ischaemic penumbra through sustained activation of postsynaptic receptors, leading to a massive influx of calcium, sodium and water into neurons. These elevations rapidly normalise on vessel opening, although secondary elevations in glutamate may occur after 2–4 hours of reperfusion.[43] So far, all the antiglutamatergic strategies have been ineffective or unsafe in patients with AIS. In a rat model of focal cerebral ischaemia, peritoneal dialysis reduced blood glutamate levels and infarct volume.[44] DIAGLUICTUS2 (feasibility and safety study to evaluate the neuroprotective effect of hemodialysis in acute ischemic stroke) is a single-centre, randomised, controlled, open-label, safety study where patients with complete reperfusion after thrombectomy are randomised to two peritoneal dialysis sessions, or to best medical treatment.

Two recent meta-analyses did not solve the controversy of whether transcranial direct current stimulation (tDCS) facilitates long-term motor recovery.[45] TESSERACT-BA (transcranial direct current stimulation as a neuroprotection in acute stroke before and after thrombectomy) is a single-centre, sham-controlled, dose-escalation, safety study where cathodal tDCS is delivered to the penumbra in patients with stroke undergoing thrombectomy. Efficacy outcome measures include penumbral salvage, collateral enhancement and infarct growth. The cellular and molecular mechanisms underlying the effects of tDCS are unclear, but the rationale of using tDCS is to facilitate the excitability of an injured and less excitable ischaemic motor cortex, and reduce the increased excitability of the contralateral motor cortex. To that aim, high-frequency repetitive stimulation (>3 Hz) cathodal stimulation is applied to the unaffected hemisphere, while anodal stimulation of the affected hemisphere would reverse the ipsilesional hypoexcitability.

Verapamil for neuroprotection in stroke is a small, phase I study aimed to replicate the safety and efficacy of intra-arterial verapamil in patients treated with thrombectomy. Furthermore, the MAVARIC (magnesium and verapamil after recanalization in ischemia of the cerebrum: a clinical and translational study) trial is a phase I, blinded-outcome, randomised, placebo-controlled study currently investigating the safety and feasibility of intra-arterial administration of verapamil in combination with magnesium sulfate immediately following successful thrombectomy. Magnesium is thought to exert its neuroprotective effects by antagonising N-methyl-D-aspartate (NMDA) signalling and limiting excitotoxicity. However, the large FAST-MAG (field administration of stroke therapy-magnesium trial)[46] showed that magnesium sulfate did not improve the primary outcome compared with placebo, including the subgroup of patients treated with alteplase.

The SONIC (safety and optimal neuroprotection of neu2000 in ischemic stroke with endovascular recanalization) trial assesses the value of Neu2000 and thrombectomy in patients with moderate-to-severe stroke (at least NIHSS ≥8) secondary to a proximal vessel occlusion. Neu2000 is an inhibitor of the Ca^{2+} permeable NMDA receptor that functions as a moderate NR2B-selective antagonist, and a scavenger of reactive oxygen

species. The inclusion of patients was terminated in July 2020 and the trial is pending final analysis.

OTR4132-MD is made of polymers designed to replace degraded heparan sulfates and foster restoration of the natural architecture of the extracellular matrix. The compound has been scarcely explored in preclinical models of stroke. Yet, the MATRISS (study to assess the safety of ReGeneraTing Agent (OTR4132) in patients with acute ischemic stroke) trial is exploring the value of a single intra-arterial injection of OTR4132-MD if thrombectomy is successful within 6 hours of stroke onset.

Imatinib is a tyrosine kinase inhibitor that inhibits ATP binding, preventing phosphorylation and the subsequent activation of growth receptors and their downstream signal transduction pathways. In mice, imatinib attenuated global ischaemia-reperfusion-induced cerebral injury by activation of JAK2/STAT3 signalling pathway along with the increase in the expression of connexin 43. At a lower than anticipated recruitment rate, the Imatinib in Acute Ischaemic Stroke Trial is investigating whether this compound initiated within 8 hours of symptom onset and given for 6 days improves the functional outcome and reduces intracerebral haemorrhage and oedema in patients with stroke treated with alteplase and/or thrombectomy.

Dl-3-n-Butylphthalide (NBP) is a synthetic compound based on l-3-n-butylphthalide that is isolated from seeds of *Apium graveolens* and it is claimed to reduce oxidative damage, inhibit platelet aggregation and improve mitochondrial functions.[47] Currently, the AIS (butylphthalide in adult patients with acute ischemic stroke) trial, BAST (efficacy and safety of Butylphthalide for acute ischemic stroke patients receiving intravenous thrombolysis or endovascular treatment) trial and NCT03394950 are exploring the safety and efficacy of NBP in patients with AIS who receive alteplase and/or thrombectomy.

Hyperglycaemic is associated with worse clinical outcomes in patients with AIS and a number of trials are exploring different compounds to counteract the toxicity of excessive glucose. In the SHINE (stroke hyperglycemia insulin network effort) trial,[48] treatment with intensive versus standard glucose control for up to 72 hours did not result in a significant difference in favourable functional outcome at 90 days. These findings do not support using intensive glucose control in this setting, unless other complementary therapeutic measures are put in place. Indeed, the significant clinical benefits of UA administration in hyperglycaemic patients[33] strongly suggest that blockage of excessive oxidative stress, and not only glucose control, might be a necessary condition to limit glucose toxicity in AIS. Other approaches to limit the effects of hyperglycaemic are ongoing, including the administration of exenatide, a synthetic glucagon-like peptide-1 receptor agonist that increases insulin secretion. Thus, TEXAIS (trial of exenatide in acute ischaemic stroke) is a multicentre, prospective, randomised, open-label, blinded endpoint (PROBE) trial comparing exenatide to standard of care given within 9 hours of stroke onset. In patients receiving tPA, exenatide is given alongside, or as soon as possible, following tPA administration, and may include thrombectomy. Glibenclamide (US adopted name, glyburide), causes the closure of K-ATP channels and results in membrane depolarisation in the pancreatic β-cell leading to increased insulin secretion by directly binding to the sulfonylurea receptor 1 of subunits of these channels.[49] SE-GRACE (safety and efficacy of glibenclamide combined with Rt-PA in acute cerebral embolism) evaluates the safety and efficacy of glibenclamide versus placebo in patients treated with intravenous alteplase within 4.5 hours after stroke onset while the target time from symptom onset to the start of study drug is ≤10 hours. The CHARM (intravenous glibenclamide for severe cerebral edema following large hemispheric infarction) trial is also assessing the value of glibenclamide in patients with large hemispheric infarction. Previously, glibenclamide was well tolerated in GAMES-RP (glyburide advantage in malignant edema and stroke trial: rationale and design), although there was no difference in the composite primary outcome (proportion of patients with a mRS score of 0–4 at 90 days without undergoing decompressive craniectomy).[50]

JPI- 289 is a novel water soluble PARP-1 inhibitor that reduced Poly (ADP-ribose) polymerase (PARP) activity, increased ATP and NAD+ levels and decreased apoptosis-associated molecules in vitro and in culture cells. Massive DNA damage after stroke elicits hyperactivation of PARP-1, causing rapid depletion of the intracellular nicotinamide adenine dinucleotide (NAD) and ATP pools, slowing the rate of glycolysis and mitochondrial respiration and leading to cellular necrosis and apoptosis. The pharmacological inhibition of PARP-1 activity to limit cellular injury and improve the outcome of cerebral ischaemic injury in animal stroke models has been studied. A Korean trial is investigating in patients with large vessel occlusions that recanalise (mTICI ≥2b) after thrombectomy, whether JPI-289 is superior to placebo to limit infarct growth.

CONCLUSIONS

The clinical stage of neuroprotection in AIS is full of neutral or negative results. This review does not provide a systematic analysis collating all empirical evidence gathered in the field of neuroprotection in patients with stroke treated with reperfusion therapies, nor can it offer an infallible prediction of the future in the field. However, the review summarises what in the authors' view are the most exciting results gathered in the last 5 years including the clinical benefits obtained in important patients' subgroups with compounds such as UA (women, hyperglycaemic, thrombectomy) or nerinetide (thrombectomy without alteplase). The review also describes strategies and drugs currently explored in RCTs. Overall, a deeper knowledge of the ischaemic death process at the neurovascular unit, an improved preselection and evaluation of drugs at the preclinical stage as it is carried out in the SPAN project, and the ongoing and future testing of the putative neuroprotectants in patients who receive reperfusion therapies, might prove to reverse a negative situation that has lasted already too long.

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Correction: The future of neuroprotection in stroke

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