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Neuroprotection for ischaemic stroke: Current status and challenges

Antonio Moretti, Federica Ferrari, Roberto F. Villa *

Laboratory of Pharmacology and Molecular Medicine of Central Nervous System, Department of Biology and Biotechnology, University of Pavia, Italy

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ABSTRACT

Stroke is the third cause of death worldwide and the main cause of chronic, severe adult disability. We focus on acute ischaemic stroke, which accounts for approximately 80% of all strokes. The current therapy aims at restoring cerebral blood flow within a narrow time window in order to prevent damaging the "penumbra" which surrounds the infarct core. Intravenous thrombolysis remains the fundamental treatment worldwide, though not ideal for various restrictions and complications, limiting to 10% or less the percentage of patients treated within the appropriate time window.

Neuroprotection is an alternative or adjunct approach to thrombolysis, targeting cerebral parenchyma in the acute ischaemic phase. Furthermore, neurorepair attempts to restore neuronal function in the after-stroke phase in those patients (treated or untreated) with significant impairment.

In the past decades, the efficacy and safety of numerous candidate neuroprotective agents were shown in various animal stroke models. However, in clinical trials, promising pre-clinical studies have not been translated into positive outcomes. Our review will analyse the possible reasons for this failure and the new approaches and recommendations to overcome it, as well as novel strategies targeting additional events in ischaemia cascade. The combination of thrombolysis with pharmacological and non-pharmacological neuroprotective approaches has also been tested. Finally, the neurorepair strategy will be described with special emphasis on the role of cell-based therapies and ischaemic conditioning.

Hopefully, the future therapy of ischaemic stroke will encompass a combination of neuroprotection (to stabilise penumbra), thrombolysis, antithrombotics (for secondary prevention) and neurorepair based on cell therapy plus rehabilitation.

Contents

Abbreviations: AIS, acute ischaemic stroke; BBB, blood brain barrier; CBF, cerebral blood flow; EPO, erythropoietin; G-CSF, Granulocyte-colony stimulating factor; i.a., intra-arterial; i.v., intravenous; IPC, ischaemic preconditioning; iPSCs, induced pluripotent stem cells; MCA, middle cerebral artery; MCAO, middle cerebral artery occlusion; MMP-2, MMP-9, matrix metallo-proteinase-2, 9; MRI, magnetic resonance imaging; MSCs, multipotent mesenchymal stromal cells; NADPH, reduced nicotinamide adenine dinucleotide phosphate; NIHSS, National Institutes of Health Stroke Scale; NINDS, National Institute of Neurological Disorders and Stroke; NMDA, N-methyl-D-aspartate; NOX, NADPH oxidase; RCT, randomised clinical trial; rIPC, remote ischaemic preconditioning; rIPerC, remote ischaemic perconditioning; rIPostC, remote ischaemic postconditioning; ROS/RNS, reactive oxygen species/reactive nitrogen species; rt-PA, recombinant tissue plasminogen activator; SICH, symptomatic intracranial haemorrhage; STAIR, Stroke Therapy Academic Industry Roundtable.

* Corresponding author at: University of Pavia, Department of Biology and Biotechnology, Laboratory of Pharmacology and Molecular Medicine of Central Nervous System, Via Ferrata 9, 27100 Pavia, Italy. Tel./fax: +39 0382 986391.
E-mail address: robertofederico.villa@unipv.it (R.F. Villa).

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1. Introduction

Few pharmacotherapies have drawn the attention of medical circles and have been debated as much as thrombolytic therapy for acute ischaemic stroke (AIS). To date, the only approved thrombolytic agent is the recombinant tissue plasminogen activator (rt-PA) firstly found to be effective in the large randomised trial of the National Institute of Neurological Disorders and Stroke (NINDS, 1995). Eighteen years after being approved by the Food and Drug Administration (FDA), i.v. rt-PA remains the fundamental therapy for recanalisation in AIS patients worldwide (review in Balami et al., 2013a), though far from ideal because of various restrictions in using it and complications. This emphasises the need for safe and effective neuroprotective drugs to be proved in accurate randomised, controlled, double-blind clinical trials.

The last three decades have witnessed a new surge of basic science investigations on the pathophysiological events following AIS. The elucidation of their mechanisms at vascular, cellular and molecular levels led to the identification of several potential targets for neuroprotection and neurorepair. Although so far clinical trials have repeatedly failed, neuroprotection is still a promising option for AIS treatment.

The objectives of this review are threefold: (i) to examine the current status of neuroprotection and neurorepair for AIS; (ii) to evaluate the reasons for the failure in translation from "bench to bedside", and the possible approaches to overcome this situation; (iii) to analyse the future prospects of neuroprotection and of the modulation of neural plasticity to obtain neurorepair. We searched for experimental and clinical studies, reviews and meta-analyses published in English from 1985 to May 2014 on PubMed and Medline.

2. Neuroprotection

Neuroprotection defines any strategy which directly targets the brain parenchyma with the aims of antagonising the harmful molecular and cellular events responsible for the ischaemic damage, allowing brain cells to survive to reduced CBF and to stabilise penumbra. Therefore, neuroprotection differs from clot-removing therapies targeting the cerebral vascular system (thrombolytics, antithrombotic).

Moreover, the endogenous attempt of the injured brain to repair the structural and functional damage in the days and weeks following AIS might provide the opportunity for a *neurorepair* approach based on the increasing comprehension of the neurobiology of neuroplasticity, synaptogenesis and neurogenesis.

Due to the complexity of the ischaemic cascade, numerous molecular targets have been tackled in order to achieve neuroprotection. They include various mechanisms of excitotoxicity (glutamate release and receptor activation), calcium influx, ROS scavenging, nitric oxide production, inflammatory reactions and apoptosis (reviews in Ginsberg, 2008; Sahota & Savitz, 2011; Minnerup et al., 2012; Sutherland et al., 2012). Over the past two decades, this has produced a vast body of experimental literature on the effects of numerous potential neuroprotective agents in various in vitro primary and secondary cell lines and in vivo in animal models of focal and global brain ischaemia. These studies proved the feasibility of experimentally effective neuroprotective drugs.

3. Neuroprotective agents: experimental and clinical investigations

A list of the main promising pharmacological and non-pharmacological neuroprotectants under investigation, their mechanisms and the references pertinent for these mechanisms are shown in Table 1.

On hospital admission, fever has been associated with poor prognosis across multiple outcome measures. *Hypothermia* might not only prevent the effects of hyperthermia, but is also a potentially promising neuroprotective therapy endowed with a multi-faceted mechanism of action on the ischaemia-induced pathophysiological events (Table 1) (reviewed by Froehler & Ovbiagele, 2010; Campos et al., 2012a; Yenary & Han, 2012). The clinical indication for the use of hypothermia in stroke patients stems from the proven therapy of coma following cardiac arrest and neonatal hypoxic–ischaemic encephalopathy. Experimentally, even a modest cooling (34 °C/35 °C for at least 2 h) showed consistent benefit in various animal stroke models, albeit with methodological heterogeneity suggesting caution in the interpretation of the results (van der Worp et al., 2007). In stroke patients, hypothermia is currently achieved by either surface cooling or by endovascular methods using catheters introduced in the inferior vena cava. There are a number of unresolved practical issues (depth, timing, duration of cooling, control of rewarming) and adverse events: shivering, pneumonia and haemodynamic effects. Pharmacological control of shivering can be achieved with dexmedetomide, buspirone, meperidine. Unfortunately, the small, preliminary clinical studies provided no evidence of beneficial effects in terms of mortality or dependency (meta-analyses and reviews in: den Hertog et al., 2009; Lakhan & Pamplona, 2012).

The Phase 1 pilot study ReCCLAIM I (Horn et al., 2014) and two randomised trials (Hemmen et al., 2010; Piironen et al., 2014) showed the feasibility of combining hypothermia either with intravenous or intra-arterial thrombolysis. In a recent, prospective cohort study, hypothermia reduced cerebral oedema and haemorrhage and was associated with better neurological outcome with no different complication rate (Hong et al., 2014). However, other reports also found that: (i) low body temperature is associated with stroke severity in patients receiving rt-PA (Kvistad et al., 2012), (ii) patients with high body temperature have increased benefit from the treatment with rt-PA, a finding possibly related to the in vitro greater rt-PA enzymatic activity at higher temperature (de Ridder et al., 2013). Two ongoing Phase III large multicentre RCTs (ICTuS-2/3, EuroHYP-1) (van der Worp et al., 2014) should establish whether hypothermia is beneficial or futile in AIS. Moreover, intra-carotid infusion could open a more rapid and safer avenue for acute hypothermia-neuroprotection than the systemic administration (Choi et al., 2010).

Several mechanisms of action have been reported for *magnesium sulfate*, a neuroprotective agent in MCAO model in rodents (Table 1); (reviewed by Westermaier et al., 2013). Dietary magnesium intake has been inversely associated with risk of stroke. A previous, large multicentre RCT on intravenous $MgSO_4$ did not report beneficial effects, a finding attributed to the delayed drug delivery within 12 h from stroke onset [Intravenous Magnesium Efficacy in Stroke (IMAGES) Study Investigators, 2004]. Subsequently, the observation that the efficacy of neuroprotective agents rapidly declines over time prompted an investigation on early, prehospital $MgSO_4$ administration in suspected stroke patients. FAST-MAG was a large, multicentre, randomised, double-blind, placebo-controlled, pivotal phase III trial that enrolled ambulance-transported patients within 2 h after stroke onset (Saver et al., 2014a,b). It was also the first to deliver early a neuroprotective drug capable of preserving larger areas of ischaemic penumbra within the "golden hour" (Saver et al., 2010), the time when brain tissue can still be salvaged. Regrettably, recent results did not show the superiority of magnesium treatment over placebo. However, they lent solid support to the practicability of prehospital trials thanks to a dedicated, widespread organisation (Saver, 2014).

Serum *albumin* concentrations are inversely associated with increased stroke risk. In experimental studies on ischaemic stroke in rodents, human intravenous albumin was a promising neuroprotectant as shown by: (i) decreased infarction volume and improved neurological score (Belayev et al., 2001), and (ii) enhanced CBF both in the ischaemic core and in the penumbra, an effect which was related to the extent of collateral flow (DeFazio et al., 2012). This observation has clinical relevance because of the role of collaterals in the clinical outcome of AIS patients.

Unfortunately, both part 1 and part 2 of ALIAS (an international RCT on human albumin) were halted prematurely because albumin