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TRANSLATIONAL CHALLENGES OF NEUROPROTECTION STRATEGY IN ISCHEMIC STROKE

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Abstract

Neuroprotection is a therapeutic strategy that attempts to save neurons from irreversible injury by modifying the effects of the ischemic cascade or facilitating reperfusion. Although numerous agents have shown neuroprotective effect in preclinical trials, their translation to clinical trials failed to show any meaningful effect. The Stroke Therapy Academic Industry Roundtable (STAIR) guidelines were made for performing research on neuroprotective agents in pre-clinical and clinical trials. Although the STAIR guidelines have been available for more than ten years, we still do not have any adequate neuroprotective agents. Reasons for unsuccessful translation from preclinical to clinical research can be considered along stages of drug development: 1) preclinical, 2) transitional and 3) clinical. By extending the therapeutic window for application of intravenous thrombolysis in acute stroke patients to 4.5 hours, as well as increasing the use intra-arterial thrombolysis and development of mechanical devices for thrombectomy in 6 hour period we may be able to achieve some degree of neuroprotection in acute stroke. Future therapy is likely to add to the current thrombolytic therapy with potential neuroprotective drugs or procedures.

Keywords

• Ischemic stroke • Neuroprotection • Translational problems

Introduction

Stroke is the third cause of mortality and leading cause of disability worldwide [1] and recently has been accepted as a medical emergency [2,3]. Generally stroke can be classified to hemorrhagic (40% of total cases) and ischemic (60% of total cases) [4]. The TOAST classification classifies ischemic stroke as a result of large-vessel atherosclerotic disease, small-vessel atherosclerotic disease, a cardioembolic source, other determined etiologies, and undetermined or multiple possible etiologies [5]. Neuroprotection is a therapeutic strategy that attempts to save neurons from irreversible injury [6-8].

Thrombolysis with tissue plasminogen activator is the only approved drug for the treatment of acute phase of ischemic stroke [9-11]. Before the thrombolysis era, clinical trials in stroke prevention were more successfull than trials in acute stroke treatment, in which neuroprotecion trials are included [12,13]. In last two decades, neuroprotection drug research has dramatically increased [14-16]. Preclinical research on many pharmacological agents with different mechanisms of action showed success in neuroprotection on animal stroke models [17]. Yet in translation from preclinical to clinical randomized trials, these agents failed to demonstrate a neuroprotective effect [6,18]. In response to numerous clinical trials on drugs in neuroprotection that have failed to demonstrate adequate effect, the Stroke Therapy Academic Industry Roundtable (STAIR) guidelines were developed in order to provide a structured process and standards for carrying out research on neuroprotective agents in pre-clinical and clinical trials [19-21]. However, despite enormous efforts and the fact that the STAIR guidelines have been available for more than ten years, no effective neuroprotective treatment is available in ischaemic stroke [15].

The aim of this review is to present the review of the current status of neuroprotection in ischemic stroke, to point out reasons for failure of neuroprotective agents in clinical practice and to show possible future paths of development of the research and the clinical use of potential neuroprotective agents or procedures.

Ischemic cascade and reperfusion injury

Ischemic cascade and reperfusion are the main mechanisms that are of interest in treatment of ischemic stroke. Brain ischemia initiates an "ischemic cascade", a complex sequence of metabolic events beginning with energy depletion that involves the generation of nitrogen oxide and free radicals through the excitation of NMDA (*N*-methyl-D-aspartate) receptors and intracellular influx of calcium, finally resulting in the induction of apoptotic and necrotic pathways. All these metabolic events occur within a few minutes or hours after the ischemic process and therefore may be potential sites of possible action of a neuroprotective agent [12,22,23]. Figure 1 shows a schematic of the ischemic cascade.

For many years it was thought that the reperfusion could prevent ischemic damage of brain in stroke, but often reperfusion damage

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causes additional brain tissue damage in ischemic stroke [24]. Reperfusion encourages leukocytes migration with activation of inflammatory response, release of cytokines, increased expression of leukocyte adhesion molecules on endothelium surface, occlusion of small vessels, and worsening of the ischemia [25,26]. Also, the formation of free radicals causes mitochondrial damage that activates proapoptotic proteins that begin the programmed cell death of apoptosis. In addition to these effects, free radicals damage the DNA causing further injury to the cell membrane causing cell necrosis [27]. During the inflammatory response, phagocytes eliminate the "healthy" cells and contribute to the further free radical formation [25]. Figure 2 summarizes mechanisms of the reperfusion injury.

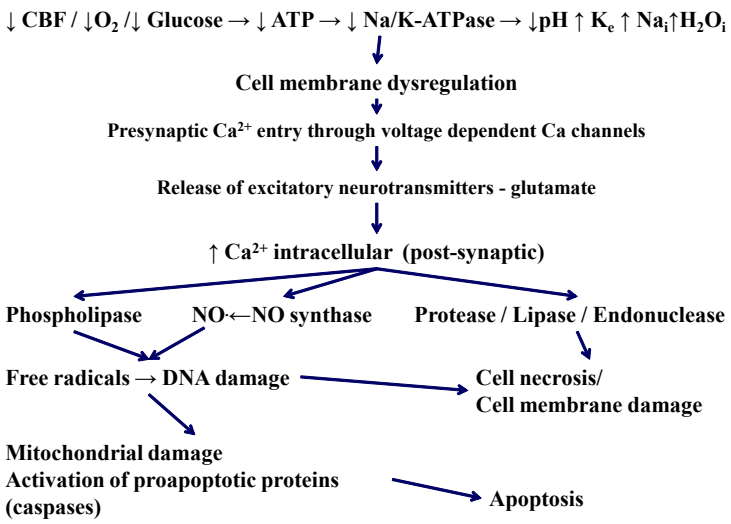

Figure 1. Schematic representation of the ischemic cascade.
Modified from reference [55]. Abbreviations: CBF - cerebral blood flow, O_2 – oxygen, K_e – extracellular potassium, Na_i – intracellular sodium, H_2O_i – intracellular water, Ca^{2+} – calcium, NO· – nitric oxide.

Preclinical animal models in neuroprotection

Preclinical research can be carried out using in vitro and in vivo models. *In vitro* studies use neuronal or mixed cell cultures and organotypic slice preparations as model systems that recreate some of the consequences of a focal ischemic insult [28]. The three main classes of animal stroke models are global ischemia, focal ischemia, and hypoxia/ischemia (the latter exclusively in young animal). These animal stroke models can be investigated in permanent or transient cerebral arteries occlusion. The most frequently used animals in these investigations are rodents (lissencephalic species) because their cranial circulation is similar to that of humans [27-29]. The next step in drug development is testing the potential neuroprotective drug on gyrencephalic species such as dogs, pigs, and non-human primates before testing on humans [21,28].

Global ischemic insults in animal models are most commonly produced by multiple vessel occlusions, and less commonly by complete brain circulatory arrest. This model is useful in researching cardiac arrest, severe hypotension or peripheral hemorrhage, strangulation or drowning. It should be noted that the recovery from transient global ischemia may give us important data in identification of the pharmacological action of an investigated neuroprotective agent. The most widely used

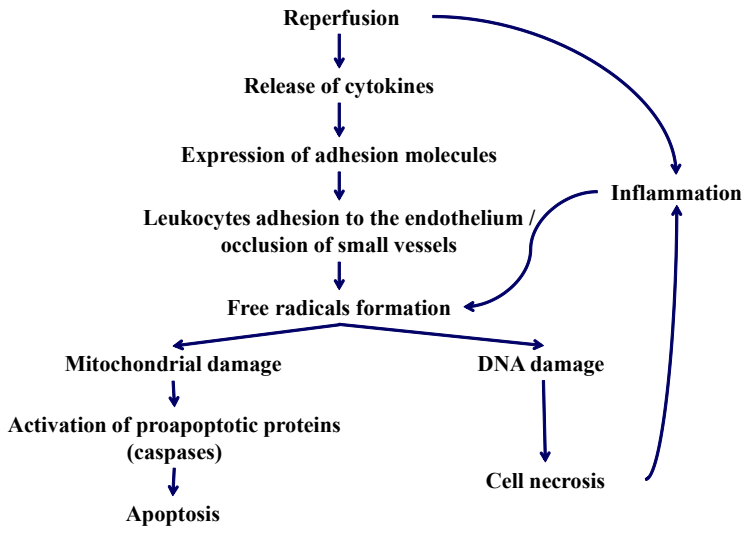

Figure 2. Schematic representation of the reperfusion injury mechanism.
Modified from references [11] and [47].

models for global cerebral ischemia in rats are four-vessel occlusion (4-VO) or two-vessel occlusion (2-VO) with hypotension, and in gerbil and mouse models, two- vessel occlusion (2-VO) [28,29].

Focal ischemia is mostly caused by a transient or permanent occlusion of the middle cerebral artery (MCA). The MCA can be occluded by electrocoagulation, an intraluminal filament, topical or intraparenchymal endothelin-1, a mechanical device such as a clip, or an autologous blood clot. A branch of MCA can be occluded by intravascular thrombin injection or photochemical occlusion.

Embolic models of focal ischemia are divided into two main categories: 1)

embolization induced by the introduction of blood clots or artificial emboli [30,31] and 2) local chemically-initiated thromboembolism [28,29].

Major preclinical outcome measures for pharmacological stroke drug testing are the size of an infarction lesion and neurological deficit. Infarct volumes are quantified by histologically stained brain sections or by magnetic resonance imaging (MRI). Brain swelling measurements are also taken into account despite the size of infarct. Regional blood flow can be measured using a laser Doppler flowmetry [28,29]. The neurological assessment of an animal model is usually evaluated by the five point scale neurological deficit score or by the new 14 point neurological scoring system [28,32,33]. Further useful measurements are blood-brain barrier function, leukocyte and platelet adhesion, cell activation and adhesion molecules expression, and protein and mRNA levels [28,29].

Clinical trials in neuroprotection in ischemic stroke patients

Therapeutic strategies for neuroprotection aim to modify effects which occur in ischemic cascade and possibly achieve reperfusion [34]. The neuroprotecion effect is mostly targeting neurons in penumbra [22,35]. Their role is not simply to protect individual neurons, but also, more importantly, to protect the neurovascular unit comprised of the neuron and the supporting glial and vascular cells within its immediate environment (such as astrocytes, pericytes, microglia, oligodendrocytes, and endothelial cells of microvessels). Studies on animal models showed that neurons in penumbra have the ability to recover for up to four hours from the onset of ischemia [6]. Neuroprotective drugs and procedures which have been investigated can be divided according to mechanism of action into: 1) limitation of ischemic cascade, 2) glutamate-mediated excitotoxicity, 3) vascular-targeted therapeutics, 4) anti-inflammatory therapy, 5) other (e.g., hypothermia, hemicraniectomy, hemodilution, etc) [9,36]. Table 1 presents a simplified classification of neuroprotective drugs and procedures divided according to the main mechanism of action with efficacy which was shown.

Numerous clinical trials that have tested neuroprotection as therapy for stroke have failed to show adequate therapeutic effect and accordingly, have not gained recommendation by any neurological society (e.g., the European Stroke Organization, the American Heart Association) for routine practice use [12,36,37].

STAIR guidelines

In response to unsuccessful developments of effective neuroprotective agents despite increased research, STAIR sought to create recommendations on preclinical development of acute ischemic stroke treatment [21]. The STAIR meetings bring together academic physicians, industry representatives and regulators to discuss ways to enhance the development of acute and restorative stroke therapies. From 1999 to 2011, seven STAIR meetings were held and proposed recommendations for preclinical evaluation of stroke treatments, Phase II and III trial design, enhancing trial implementation and completion, and development of novel approaches for measuring outcome and regulatory considerations. [19-21,38-42]. The main goals of the seventh STAIR meetings were to maximize use of the intravenous thrombolysis within 4.5 hours as well as to enhance the research on mechanical devices for intra-arterial recanalization with a focus on mechanical reperfusion. Priorities for the neuroprotective/adjunctive therapy development according to these guidelines are: 1) determination of the efficacy of treatment before versus after ischemia, 2) development of plurifunctional agents or therapies that target multiple mechanism pathways, 3) development of reperfusion injury measuring techniques and therapies, 4) determination of efficacy of selective cerebral delivery (catheter-based intra-arterial delivery), 5) determination of the roles that the immune and cardiovascular systems play in neuroprotective repair mechanisms, 6) determination of efficacy of selective induction of cerebral hypothermia [42,43].

Reasons for unsuccessful development of neuroprotective drugs

Reasons for unsuccessful translation from preclinical to clinical research can be divided by the testing stages of drug development: 1) preclinical, 2) transitional and 3) clinical [36].

Among the preclinical factors which contribute to the possible failure of development of neuroprotective drugs are 1) lack of multiple investigations of various stroke models in testing therapies focus on early evaluation of outcome while ignoring later evaluations outcome that are more important to clinical investigation [9,44]. In addition, the clinical trials were performed despite failure of the preclinical trials to show evidence of the drug's neuroprotective effect [15]. Further, animal models do not fully mimic a clinical situation, since the experimental artificial stroke model differs from human stroke [45]. Studies on animal models are performed often on young animals in controlled conditions with tightly regulated temperature and blood pressure, as well as possible control over the severity of an ischemic lesion [9,44,46]. In early research in neuroprotective drug development, treatment was administered immediately before or within a short time of the insult [44]. These measurements cannot be reproduced in human trials, and therefore, outcomes in animal studies cannot accurately predict outcomes in human studies.

There are numerous other possible explanations for the failures. For example, rats and gerbils have different gray/white matter ratio with higher blood flow and metabolic rate than human brains. The statistically significant, albeit physiologically modest experimental neuroprotective effect, diminishes under human conditions (aging, multiple brain comorbidities such as arteriosclerosis, hyperglycemia, dyslipidemia, etc.). Moreover, the animal models mostly use territorial infarct models with healthy vasculature, and in normal ranges of glucose concentrations and blood pressure, while in the clinical realities, both lacunar and territorial, forebrain and vertebrobasilar teritories may be concurrently affected [28,29].

Table 1. Simplified classification of neuroprotective drugs and procedures according to the main mechanism of action.

Mechanism of neuroprotection	Mechanism of drug/ procedure acting.	Neuroprotection efficacy (Clinical investigations)	Reference
Limiting ischemic cascade	Calcium channel blocker (nimodipine, flunarizine)	No efficacy	[9,36,53,56-59]
	Sodium channel blocker (lubeluzole, fosphenytoin)	No efficacy	[7,36,53,60]
	Potassium channel modulators (BSM 204352)	No efficacy	[9,36,53]
Preventing excitotoxicity	NMDA antagonists (dekstrophan, selfotel, aptiganel)	No efficacy	[9,13,36,53,61,62]
	AMPA antagonists (NBQX, YM-872)	No efficacy	[3,36]
	Antagonists of glycine regulatory sites (Gavastinel)	No efficacy	[3,36]
	Non-NMDA receptors modulators • Opioid receptors antagonist nalmephen • GABA agonist – clomethiazol • Serotonin agonist - repinotan	No efficacy	[36,53,64,65]
	Magnesium	In progress (FAST-MAG)	[6,9,36,51,52]
Anti-inflammatory drugs	Anti-adhesion molecule therapy (antibodies) • Enlimomab – anti-ICAM1 • LeukArrest -Hu23F2G – antileukocyte antibody • Abcixmab – antitrombocyte antibody	No efficacy	[9,36,51,53,66,67]
Free radicals scavengers	Tirilazad	No efficacy	[9,36,53,68]
	NX-059	No efficacy	[9,36]
Other agents and procedures	Citicoline	In progress (ICTUS)	[9,15,51,52]
	Hypothermia	In progress (CHILLI)	[6,15,54,70]
	Hemodilution – albumins	In progress (ALLIAS II)	[6,15,51,52]
	Minocycline	No efficacy	[51]
	Fibroblast growth factor	No efficacy	[70]
	Statins (pravastatin, lovastatin)	In progress (Neu-START II)	[36,51,52]
	NeuroFlo device	No efficacy	[51-53]
	Transcranial laser technology	In progress (NEST-3)	[51,52,54]

The most influential factor affecting the transitional stage is the genetic variability in humans. In animal models, genetic variability is highly constrained, and therefore possible drug responses are limited according to these constraints. Because of this, we cannot predict side effects that may be produced in humans by drug doses used in experimental models [14,47]. Another factor that may be operational is that the common use of anesthetics in preclinical trials affect outcomes, as they are administered in too small of therapeutic window to be feasibly applied to most patients [47].

A main factor during the clinical stage that have an impact on the failure of neuroprotective agent are inadequate study design such as too small of a sample size of patients or a combination of different stroke models in the same study despite therapy being effective only against one type of the stroke. Other factors include the lack of standardation of outcome measures, and failure to achieve adequate effect and concentration of investigation drugs in plasma [15,47]. Analyses often did not include confounders, such as co-morbidities, age and gender [34]. A newly-emerging challenge is to consider testing potential neuroprotective therapies in patients who have received intravenous thrombolysis (IV - tPA) to study the dynamic relationship of the two therapies [43].

Further perspective of clinical trials in neuroprotective agent development

The extension of a therapeutic window for application of intravenous thrombolysis in acute stroke patients for 4.5 hours, as well as

increasing the use intra-arterial thrombolysis and development of mechanical devices for thrombectomy in six hour period gives a chance for neuroprotection in acute stroke [40]. But emphasis must be given to the crucial point that both the recanalization and neuroprotective treatment be administered in acute stroke [48-50].

Despite previous failures, clinical trials in neuroprotective agents are continuing. The most promising neuroprotective drugs and procedures are citicoline, hypothermia, magnesium, albumins and statins [15,51].

Citicoline showed a robust neuroprotective effect in preclinical studies, but a mild neuroprotective effect in clinical trials, which was recognized by ESO [12], but not recommended by the neurological professional societies [12,36,37]. We are waiting for the results of the *International Citicoline Trial on Acute Stroke* (ICTUS), in which patients are still being recruited [15,51,52].

Hypothermia has shown a neuroprotective effect in cardiac arrest and neonatal encephalopathy [15]. The *American Heart Association* (AHA) guidelines suggest that the hypothermia could be an option as a supporting strategy in reperfusion therapy [36]. The *Controlled Hypothermia in Large Infarction* (CHILI) study is currently recruiting patients with a large anterior circulation ischemic strokes [6,15,52].

Despite the neuroprotective effects magnesium shown in preclinical trials, improving vasospasm in subarachnoidal hemorrhage has shown neuroprotective effects as well. The *Field Administration of Stroke Therapy – Magnesium Phase III Trial* (FAST-MAG) is currently being carried out, in which hyperacute ambulance-initiated magnesium therapy is being investigated in patients with acute stroke [6,41,52].

Albumin Therapy for Neuroprotection in Acute Ischemic Stroke (ALLIAS II) trial [6,51,52] investigates high doses of human albumins with multimodal actions on neuroprotection [15].

A high dose of lovastatin is currently investigated in *Neuroprotection with Statin Therapy for Acute Recovery Trial* (Neu-START II) [51,52].

Recently published trial *Safety and Efficacy of NeuroFlo Technology in Ischemic Stroke* (SENTIS) did not show neuroprotective benefit from the use of a partial occlusion of abdominal artery by NeuroFlo catheter [51-53]. Further studies are expected from promising transcranial laser technology treatment in *Efficacy and Safety Trial of Transcranial Laser Therapy Within 24 Hours From Stroke Onset* (NEST-3), as the pooled analysis of previous two trials (NEST-1 and NEST-2) revealed significantly improved treatment success rate in patients treated with laser therapy [51,52,54].

Despite all these efforts even by the STAIR guidelines directing preclinical and clinical investigations to focus on key problems in research on development of drugs or procedures, there is still no effective neuroprotective agent for ischemic stroke [40]. A possible solution for the high-risk patients is preloading with an experimentally successful drug that can amplify the neuroprotective effect and solve the problem of *a posteriori* transfer of the drug to a insufficiently perfused tissue. This theory is supported by the comparison, as the majority of drugs used in trials had significantly higher efficacy when given before than after the ischemic event.

It is likely that the current thrombolytic therapy will be accompanied by some of the possible neuroprotective drugs or procedures [6,8,42,43,50]. There is also a possibility that in future we would use neuroprotective agents with multimodal characteristics which are acting on multiple points of the ischemic process [14]. The positive results of currently implemented clinical trials in neuroprotection are promising, but according to past results, we should be patient. By rigorous and diligent scientific research of new possible therapies of neuroprotection, we come closer to achieving better treatment outcomes for our patients with ischemic stroke.

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