The History of Clinical Neuroprotection Failure

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Abstract: Neuroprotection in stroke treatment refers to a group of treatments and drugs aimed to antagonize the biochemical and molecular processes that lead to irreversible ischemic damage. In recent years, several clinical studies have been conducted to test the efficacy of several promising molecules with different mechanisms of action. However, the results obtained from preclinical studies (on in vitro models or on animals), despite having provided excellent results, making the goal of stroke neuroprotection at least achievable, were accompanied by a high failure rate. The reasons for these failures are linked to the unbridgeable difference between the animal and human models and to the marked heterogeneity of stroke in humans. Although future perspectives are encouraging, other techniques such as neuroprotectant cocktails, reperfusion, improving angiogenesis and collateral circulations, and infarction prevention, may represent a goal in stroke neuroprotection.

1. Neuroprotection in Stroke: Number of Molecules from Past to Present

Over the past 30 years, further research in the field of neuroprotection has been conducted. Neuroprotection could be defined as any strategy applied to antagonize molecular and cellular events that lead to ischemia, targeting brain cells to improve their survival [1].

First studies emerged from the 1970s, but the crucial development occurred in the 1990s and 2000s when the basis of ischemic damage was discovered. Excitotoxicity, caused by a reduction in blood flow, was the first mechanism of brain injury, which helped the comprehension of many underlying processes and the detection of relevant therapeutic targets for ischemic stroke [2]. There was a growing sense that ischemic stroke was not only a vascular disease, but many vascular and neural cells such as astrocytes, macrophages, neurons, and endothelial cells formed a unique entity involved in the damage [3]. These results suggest that brain injury was not only the product of local alteration, but many systemic mechanisms could be also involved [4].

Magnesium sulfate acts as a neuroprotector in the middle cerebral artery occlusion model in rodents, blocking the N-methyl-D-aspartate (NMDA) receptors, reducing glutamate release, and blocking calcium channels [5]. In 2004, a large multicenter randomized controlled trial (RCT) on the intravenous infusion of magnesium did not report any benefit; this was attributed to a delay in the administration of molecules (after 12 h from acute brain injury). In 2014, another multicenter, randomized, double-blind, placebo vs controlled pivotal phase III trial (FAST_MAG) studied

the administration of magnesium in patients within 2 hours after stroke; thus, magnesium treatment was inferior to the placebo (Table 1).

Antioxidant oxidative stress is one of the main mechanisms implicated in ischemic injury. Reactive oxygen species (ROS) scavengers have shown neuroprotective effects in preclinical models [6], even if these results have not been confirmed in clinical studies. Data on ebselen are contrasting; in a few studies [7,8], it seems to reduce brain injury due to cerebral ischemia, and RCT shows that receiving ebselen within 6 h from the event reduces the infarction and improves the functional outcome [9]. Unfortunately, a recent phase III trial did not confirm the neuroprotective action of this molecule [10]. Following the high-quality evidence shown in preclinical studies, a Cochrane review in 2011 [11] highlighted the inefficacy of edaravone. However, in 2013, a clinical study showed the efficacy of edaravone administered in combination with thrombolysis, increasing the revascularization and reducing the infarction of the lesion [12] (Table 1).

Haematopoietic growth factor: Granulocyte-colony-stimulating factor (G-CSF) and erythropoietin (EPO) reduce the excitotoxicity induced by glutamate, and also increase the neuroangiogenesis with an anti-inflammatory and anti-apoptotic action. G-CSF appears to be neuroprotective in preclinical studies where it seems to reduce the infarct size and the functional outcome [13,14]. These results are confirmed, even if the administration is delayed within 72 h [15]. Regardless, clinical trials are discouraging. In a multicenter RCT (AX200), the EV administration of G-CSF within 72 h did not lead to better outcomes compared to the placebo, in terms of NIHSS scores and mRS [16]. Likewise, a Cochrane review of 8 RCTs showed that G-CSF did not improve functional outcomes [17]. With regards to EPO, animal stroke models have pointed out the efficacy of this molecule in reducing infarction [18,19]. Clinically, RCT showed an increased risk of infarction and mortality in patients treated with EPO and r-tPA in combination [20] (Table 1).

Statins molecules, at high doses, have a neuroprotective effect in ischemic brain injury, thus improving endothelial function, vasodilatation, and antithrombotic and anti-inflammatory effects [21]. It is already known that the pre-stroke administration of statin has a functional benefit, even if the administration in combination with tPA seems to increase the risk of infarction [22]. Post-stroke statin therapy in naive patients within 72 h from acute event did not improve outcomes, as confirmed by RCTs [23]. In 2015, in a multicenter, randomized, open-label, blinded endpoint, parallel group study, no significant differences were found between the two groups for the onset of stroke and for the occurrence of adverse events [24]. In conclusion, even if the action of statins on the prevention of atherosclerotic carotid plaque is well known, the neuroprotective effect of this molecule is still debated (Table 1).

Minocycline is a tetracycline antibiotic with anti-inflammatory, anti-apoptotic, and antioxidant effects that promote neuroprotection. Preclinical studies point out the efficacy of the molecule in reducing the infarct size [25]. The administration of minocycline in combination with t-PA were found to reduce brain injury and also

the risk of infarction [26]. Clinical studies confirmed the safety of the administration of minocycline alone or in combination with tPA, even if little is known about its efficacy [27] (Table 1).

Albumin has various antioxidant effects and improves microvascular blood flow in the ischemic regions [28]. The ALIAS pilot trial showed that prognosis after the administration of albumin in combination with tPA was three times better in a high-dose albumin group compared to a low-dose group [29]. However, the analysis of the combined data from part one and two of the ALIAS trials showed that treatment with intravenous albumin, at 3 months, was associated with increased rates of adverse events such as intracerebral hemorrhage (Table 1).

Citicoline is a drug with a high capacity to enter the blood–brain barrier and an excellent safety profile [30]. This molecule plays a neuroprotective role, promoting membrane stability, and inhibiting excitotoxicity, oxidative stress, and apoptosis [31]. In preclinical studies, citicoline increased SIRT1 protein levels with concomitant neuroprotection [32]. Unexpectedly, a large multicenter European RCT (ICTUS trial) on patients treated with citicoline for 6 weeks, within 24 h from acute stroke, was stopped prematurely because no differences pointed out between citicoline and placebo groups [33]. However, a meta-analysis of acute ischemic stroke showed that patients who received the highest dose of citicoline, within the first 24 h, not treated with tPA, showed improvements [34] (Table 1).

Pioglitazone is an oral drug that reduces insulin resistance in type II diabetes [35]. A phase III trial (NCT00091949) in 2015 studied the efficacy of pioglitazone in non-diabetic patients who suffered from ischemic stroke in secondary prevention. All the participants in the study had insulin resistance and the true efficacy of the molecule in non-diabetic patients was not detectable (Table 1).

NA-1 plays a neuroprotective role in protecting neurons from excitotoxicity induced by the activation of NMDA receptors [36]. A phase III RCT (ESCAPE-NA1) evaluated the neuroprotective action of NA-1 in patients undergoing endovascular thrombectomy. NA-1 did not show any beneficial effects in patients who had good outcomes after endovascular treatment when compared with the placebo group. However, the beneficial effect of NA-1 showed in patients who did not receive endovascular treatment, with better outcomes and smaller infarction [37] (Table 1).

Hypothermia in stroke animal models seems to reduce metabolic demand, preserving energy, and decreasing glutamate and ROS with anti-inflammatory and anti-apoptotic effects. In stroke patients, hypothermia is obtained by using catheters introduced in the inferior vena cava or by surface cooling. Preliminary clinical studies point out no beneficial outcomes in terms of mortality [38,39] (Table 1).

Table 1. Common neuroprotective treatments, their mechanisms of action, and main outcomes.

Neuroprotective Factors	Mechanisms	Preclinical Outcome	Clinical Outcome
Anti excitotoxicity Magnesium sulfate [5] NA-1	blocking NMDA receptors reducing glutamate release blocking calcium channel inhibiting NMDA receptors	*Effective*	*No convincing evidence*
Antioxidant Ebselen [9] Edaravone [11]	ROS scavengers	*Effective but narrow therapeutic window/study quality issues*	*Not effective* *Increased adverse events*
Haematopoietic growth factor G-CSF [16] EPO [20]	reducing excitotoxicity anti-inflammatory and anti-apoptotic effect increasing neurogenesis	*Effective but methodological bias*	*Not effective* *Increased adverse events*
Statins [24]	inhibiting HMGCoA reductase	*Effective but study quality issue*	*Contrasting results*
Antibiotics Minocycline [25]	anti-inflammatory and anti-apoptotic effects	*Effective*	*Safe* *No data about effeicacy*
Albumin [29]	Improving microvascular blood flow	*Effective*	*Increase adverse events*
Neurovascular repair Citicoline [33]	promoting membrane stability inhibiting excitotoxicity, oxidative stress and apoptosis	*Effective*	*Contrasting results*
Pioglitazone [35]	No clear mechanism	*Effective*	*Not detectable efficacy in non diabetic patient*
Non pharmacological Hypotermia [39]	reducing metabolic demand preserving energy decreasing glutamate and ROS anti-inflammatory and anti-apoptotic effect	*Effective*	*Management difficulties* *Adverse events*

2. The Failure of Neuroprotection: From Bench to Bedside

The identification of pathways underlying cell death during ischemic damage has enabled the development of new promising neuroprotective drugs. To date, the results are linked to a complex transposition from the bench to the bedside table. This difficulty has spread pessimism about the potential role of these drugs in clinical practice [40].

Among these translational difficulties, the time of administration is crucial. In preclinical studies, neuroprotective agents are applied immediately after the mechanical occlusion of the vessel [41]. This is an unlikely condition in humans, where the exact time of symptom onset is not always known and the administration of the drug is unlikely to take place in a short time. Therefore, the administration of the agent at a variable time from the ischemic event could explain the heterogeneous response presented by patients towards the same neuroprotector.

Another difference between animal and clinical studies could relate to the affected vessels. In animal models, the vessel is closed mechanically and later

reperfused. There is a concern that the infused drug will reach the ischemic zone more quickly compared to stroke patients who continue to have vessel occlusion [41].

Finally, preclinical studies use healthy animals of similar ages (typically rodents with less than 3 months of age). Human patients vary widely in the age range and usually have a variable comorbidity pattern [42].

3. Future Perspectives

The main purpose of stroke therapy is to restore cerebral blood flow after ischemic insult; the secondary purpose is to modulate the factors that could aggravate this damage and, if possible, to repair it [43].

The first problem in stroke patients is that not all of them can be revascularized, and therefore neuroprotective agents cannot sufficiently reach salvageable tissue. Moreover, many different processes occur consequently and synergistically during ischemic cascade: excitotoxicity, oxidative and nitrosative stress, inflammation, and reperfusion processes [42].

Preclinical trials combining different neuroprotective drugs (e.g., a cocktail with anti-excitotoxicity + anti-inflammatory + antioxidant properties) with vascular reperfusion therapy could represent effective future prospects in this area of research [44,45].

Moreover, good collateral circulation (pial and leptomeningeal collaterals) may improve stroke tolerance, due to fast neurological symptom improvements after thrombolytic and thrombectomy therapies and a reduction in intracranial hemorrhage risks [46].

Several strategies that could improve collateral circulation have been investigated, but none have been applicable in clinical practice [42].

Another important aspect of restoring brain damage is to prevent the no-reflow phenomenon and hemorrhagic transformation. Drugs alone have not been shown to protect the brain–blood barrier damage caused by various mechanisms during ischemia–reperfusion injury in human hemorrhagic transformation [42].

4. Conclusions

In conclusion, the development of neuroprotective therapies in stroke patients is assuming an increasingly central role in preclinical studies and, therefore, in those of translational medicine. This is related to the fact that multitarget neuroprotectants could represent a highly promising tool for improving stroke care. Future research should take into account a comprehensive strategy including neuroprotectant cocktails, mechanisms of reperfusion, angiogenesis, collateral circulations, and the prevention of post-ischemia hemorrhages.

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