Therapy of stroke – from experimental studies to clinical trials

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Abstract:
Ischemic stroke (IS) kills 5.5 million people each year worldwide and is the leading medical cause of neurological disability in adult population. Despite many years of active research for effective therapy of IS, acetylosalicylic acid (ASA) and recombinant tissue activator (rt-PA) remain the only approved medications. ASA has weak effect but can be used in almost all patients with IS. According to very strict inclusion criteria, rt-PA can be used only for small number of patients and its use is limited by hemorrhagic complication. Effective, well-tolerated treatments with extended therapeutic window are needed to decrease disability and improve patients’ prognosis. The idea of neuroprotective agent is that it interferes with mechanisms involved in ischemic cascade, among many others, glutamate release, cell calcium overload and formation of free radicals.

The problem with neuroprotectants is that fine results obtained in models of ischemia are not reproducible in clinical trials and general perception of neuroprotectants is that they work in animals but they fail in humans. It is very likely that problems begin in preclinical part of evaluation.

To improve quality of experimental studies, STAIR introduced recommendations for quality of preclinical drug testing that should be fulfilled before a new agent reaches clinical trial. Appropriate animal studies should include characterization of full dose-response curve, evaluation in models of both permanent and transient focal ischemia, independent laboratory verification and ascertainment of time-window.

It should be remembered that because of previous negative trials with neuroprotectants, no such drug has been introduced to the market.

Key words:
ischemic stroke, experimental models, clinical trials, neuroprotection, thrombolysis


Introduction

Stroke is one of the leading causes of mortality and permanent disability in adults worldwide. Approximately 15 million people per year suffer of stroke, one third die and one third are left permanently disabled [35, 20]. The majority of cases (85%) are ischemic...
strokes which result from an occlusion of a major cerebral artery by a thrombus or embolism, or disturbances of circulation in small penetrating arteries with subsequent loss of blood flow, and major decrease in the supply of oxygen and nutrients to the affected region [10].

In Poland, about 60,000 of new stroke cases are noted annually. The incidence is similar to other European countries but the mortality rate is still higher than in more developed countries. This difference is probably not caused by the differences in the therapeutic management but rather by general care approach. In some societies, there is still general opinion that stroke is hopeless and incurable condition. It appeared that quality of stroke patient care is one of the factors minimizing mortality [16].

In Poland experts from National Program of Prophylaxis and Treatment of Stroke publish periodically recommendations for stroke patients management, including organization of stroke units, rehabilitation and therapy guidelines [27, 28].

**Stroke therapy**

There are several directions of ischemic stroke (IS) treatment: urgent therapy preventing subsequent neurological impairment, primary and secondary stroke prevention and symptomatic therapy to diminish complications that are not specifically but commonly occur in patients with brain ischemia.

For many years ASA was the only therapeutic option for IS. This situation has changed when recombinant tissue plasminogen activator (rt-PA) has been introduced.

Presently there are two main directions of research in the area of IS therapy. These include restoring blood circulation in compromised area and preventing biochemical changes evolving due to “ischemic cascade”, thereby minimizing subsequent degeneration [10]. All these efforts are undertaken not only to decrease mortality but, what is even more important, to decrease patients’ disability.

According to “Helsinborg’s Declaration” introduced in 1995 “time is brain”. Nowadays for treatment decision making also “penumbra is brain” and “recanalization is brain” [19]. The assumption that the area of ischemic core is surrounded by the penumbra (low blood flow) gives the hope of potential recovery either by direct re-flow or by administration of a neuroprotectant. Penumbra imaging is now becoming possible with modern neuroimaging technics such as perfusion-CT (PCT) or perfusion- and diffusion-weighted magnetic resonance imaging (PWI, DWI-MRI) [2, 19].

**Neuroprotection**

Neuroprotective agents are used to interfere with one or more of the mechanisms involved in the ischemic cascade thus limiting the tissue damage. Search for new neuroprotective agents is based on the knowledge of the main processes involved in ischemic cascade (reduction in oxygen and energy substrates), which are linked to glutamate release and calcium influx leading to cell calcium overload with the increased production of nitric oxide, protease and phospholipase activation and final formation of free radicals [10, 17].

In theory, the thesis of neuroprotective action seems to be simple. However, up till now over 1000 experimental treatments have been studied and none of them was introduced to the market. For example only in 2004, 511 drugs were under evaluation in neurodegenerative diseases (including cerebral ischemia). A majority of them were just discovered or entered experimental studies [13] and only a few were tested in clinical trials. Drug discovery and development is known to be time and costs consuming. To register a new drug, it takes on average about 12 years and costs about 1 billion USD. Lack of efficacy and adverse effects of new chemical agents are responsible for 60% of all failures but 10% of them is linked to insufficient knowledge of metabolites and pharmacokinetic properties [1].

Despite clear efficacy of many compounds in animal models of stroke, all of them subsequently failed in the clinical settings. Due to the lack of successful trials, common perception of neuroprotective drugs is that everything works in animal models but nothing works in humans [24]. There are many efforts to explain this phenomenon and to improve experimental methodology.

It is thought that some trials of neuroprotective drugs may have failed because they included the patients with many types of stroke unlikely to benefit...
from treatment. Acute stroke patients represent heterogeneous population, differing in lesion locations in the brain (hemispheres, white matter tract, brainstem, cerebellum) and in stroke mechanisms (atherothrombotic occlusion, embolization from heart or extracranial arteries, small vessel disease or other less common causes). The features crucial for successful outcome include selection of appropriate patients based on stroke type and severity, time window from onset to treatment, dosage regimen allowing rapid achievement of therapeutic drug level, outcome measures and adequate sample size big enough to detect clinically relevant treatment differences [22].

It is possible that even if clinical trial designs have been adequate, the concept or the specific agent tested may be fundamentally incorrect [22]. The animal models of ischemia (focal ischemia, global ischemia and culture methods) [24] and the clinical condition are extremely different. It is thought that focal models the most closely resemble human stroke [12] but still some studies were conducted in other models. Profile of preclinical studies conducted with neuroprotective drugs is often limited by inappropriate selection of model of ischemia, inappropriate characteristics of experimental animals that do not reflect the population of patients with IS, inadequate selection of dosage and use of outcome measures not relevant in the patient population [22, 39]. Also methodological quality of animal studies requires improvement. Many animal studies are non-randomized with lack of power calculation and uncertain reproducibility of outcome measures [29]. Functional outcome is undisputedly the primary measure of efficacy in clinical trials whereas animal studies usually rely on infarct volume [39]. Unfortunately, infarct volume does not tell whether surviving neurons are functional, dysfunctional or destined for death in a delayed fashion [8].

Apart from differences between models of ischemia and human ischemia, it appeared that adverse events of some agents (like NMDA antagonists) were present already at the drug level lower than that required for neuroprotection [9].

In attempt to improve compound selection for clinical development, the Stroke Therapy Academic Industry Roundtable (STAIR) published guidelines for conducting preclinical and clinical studies [7, 33, 34]. A consensus view suggests that more preclinical data on candidate neuroprotective drugs in appropriate animal models are needed. This includes: tests in rodent models of focal cerebral ischemia with extended recovery and subsequent studies in primate models, toxicological studies in several species of intact and stroke models, determination of an optimal route of administration, performing dose-response studies and evaluating time-window for administration [33, 34].

The criteria of Quality of Evidence in Experimental Stroke Scale were presented in Table 1 [24]. For each fulfilled point of criteria the study may gain 1 point, so the maximum score is 10. Using a scale based on the STAIR recommendations for pre-clinical testing, it seems to be clear that any medication may be considered for clinical trial only when there is both high level of experimental efficacy and a diverse body of evidence supporting its clinical application.

**Disufenton (NXY-059)** which was an extensively investigated neuroprotective agent, performed well fulfilling both criteria and gaining 10 points on the Quality of Evidence Scale. NXY-059 is a nitrone compound with free radical trapping properties. Its neuroprotective efficacy was initially demonstrated in small-animal models of transient and permanent focal ischemia (transient and permanent middle cerebral ar-
tery occlusion (tMCAO and pMCAO), and confirmed in marmoset model of pMCAO [31]. This confirmation is a key differences in the development of NXY-059 comparing to the earlier studies with neuroprotectants and is in accordance with STAIR requirements. The marmoset model was selected due to its phylogenetic similarity to humans, possibility to serve as a model of higher mental functions because marmosets have a large brain-to-body size ratio [18].

In both species, NXY-059 decreased infarct volume and reduced long-term disability. This efficacy was achieved within the time-window relevant to clinical practice. NXY-059 is the first neuroprotective agent that met all STAIR criteria and on this basis progressed to phase III clinical trials. In 2006 results from the first part were published. NXY-059 significantly improved the overall distribution of scores on the modified Rankin scale comparing to placebo. It has not improved neurological function measured on National Institutes of Heath Stroke Scale (NIHSS) or Barthel index. It is, however, very interesting that NXY-059 given together with rt-PA decreased the risk of intracerebral hemorrhages (both asymptomatic and symptomatic) [14]. Then the question arises whether NXY-059 is a real neuroprotectant or acts rather on cerebral blood flow [25]. For this agent and for many others with not so positive clinical background as for NXY-059, more rigorous preclinical study methodology may lead to more reliable and reproducible results. On the other hand, neutral results of SAINT II study, which was planned to be confirmatory, may suggest that intravenous administration of neuroprotective agent is not effective. Publication of SAINT II results is still awaited but it looks that it would be very disappointing [15].

Some ideas about the effects of neuroprotection in ischemic stroke come also from epidemiological studies showing a correlation between diet and risks of cardiovascular diseases. Polyphenols can be an example of such potentially effective therapeutic agents. They are widely distributed in the human diet, found mainly in plant-derived food and beverages and they are known to be most abundant antioxidants in the diet. It is estimated that their intake is 10 times higher than vitamin C and 100 times higher than vitamin E. In vitro studies have demonstrated that polyphenols interact with all pathological processes leading to brain tissue damage and cell death occurring during cerebral ischemia. In rat models of transient cerebral ischemia, polyphenols given orally one week before stroke induction appeared to inhibit massive release of glutamate and aspartate. The volume of brain infarct was reduced by 60% by polyphenols treatment [5]. Despite these positive features in experimental studies, polyphenols have not been introduced to clinical evaluation in IS.

Other promising factors with neuroprotective potential are neuropeptides, such as PACAP (pituitary adenylate cyclase activating polypeptide). PACAP applied intracerebroventricularly to rats before pMCAO significantly reduced the infarct volume measured 12 h and 24 h after the onset of the ischemia. Similar results were obtained when PACAP was given intravenously even 4 h after ischemia onset. The possibility of delayed administration with positive effect on ischemic tissue damage is the most promising part of the studies [6], however, it needs further verification.

It must be remembered that up till now no "pure" neuroprotectant achieved positive results in the clinical studies and none was introduced to the market. The only agents with some neuroprotective activity which have been registered and successfully used in IS are statins. Apart from inhibition of HMG-CoA reductase and limitation of cholesterol biosynthesis, experimental studies revealed that statins directly up-regulated endothelial nitric oxide synthase (eNOS), increased intracellular release and bioavailability of nitric oxide (NO) leading to enhanced cerebral blood flow and protection from cerebral ischemia [32]. Apart from experimental studies, clinical trials showed that the use of statins reduced the risk of both fatal and non-fatal IS and were recommended for stroke prevention.

**Thrombolysis**

rt-PA was shown to be effective in rat thromboembolic stroke model when given up to 3 h after infarct [3]. The drug was approved by FDA in 1996 on the basis of positive results coming from the National Institute of Neurological Disorders and Stroke (NINDS). In 2002 it was also introduced to Europe with license restricted to specialist centers.

Intravenously given Alteplase is licensed for use in IS but only within limited 3-h time window [23]. In the NINDS study, a half of the participants received
thrombolytic treatment within 90 min of the onset and the drug was found to prevent one death for every seven patients treated [23]. It must be, however, remembered that only 1–8.5% hospitalized patients may receive thrombolytic treatment due to the need of CT examination, short time window and risk of intracerebral hemorrhage [20].

Presently, thrombolytic therapy is clinically used in most countries. Poland participated in SITS-MOST registry which was designed to measure the safety and effectiveness of thrombolytic therapy when used in a 3-h therapeutic window after the onset of stroke [38]. Up till now over 312 patients have been treated with Alteplase in Poland within registered indication (up till 3 h from stroke onset).

Some efforts are made to lengthen the time window and clinical trials are conducted to prove efficacy and safety of thrombolysis within longer period of time: The Third International Stroke Trial within up to 6 h of stroke onset and ECASS 3 (European Australasian Acute Stroke Study Investigators) within 3 to 4.5 h. Patients are randomly given a standard dose of intravenous rt-PA or placebo [30].

The main concern of rt-PA therapy is increased risk of symptomatic hemorrhagic complication within the central nervous system, which occur in 2–6% of patients [23, 38]. It was revealed that in animal model and also stroke patients baseline level of some matrix metalloproteinases (MMPs), such as MMP-9, might be of value in predicting this complication, the same was demonstrated in non-thrombolytic and thrombolytic patients [4, 21, 37].

Some of the MMPs inhibitors such as BB-94 or minocycline have been already tested in small animals with cerebral ischemia treated with rt-PA [26] but their therapeutic potential in atherosclerosis awaits further investigation [36]. However, it seems that MMPs inhibitors may have therapeutic potential to improve safety profile of thrombolytic drugs.

Desmoteplase is a new thrombolytic agent that is now under evaluation. It is a genetically engineered thrombolytic protein present in the saliva of the vampire bat. It is postulated to be safer in terms of hemorrhagic complications but it is under evaluation and needs further verification [11].

It is also assumed that in the near future a series of potentially efficient interventions such as sonothrombolysis, intraarterial or mechanical thrombolysis would be used to improve the rate of successful recanalization.

### Conclusion

Stroke is still a dangerous and life-threatening condition but fortunately we are far away from the times when stroke patients were left to die or be disabled. Medicine covered a long distance in perception and management of stroke but development of safe and effective treatment is still a major challenge of experimental and clinical neuroscience. It must be remembered that drugs should be tested in clinical trials only if data from experimental studies are valid and precise. Despite huge number of clinical trials with different neuroprotective agents, none has appeared to be safe and effective enough to be introduced to the market.

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