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Neuroprotection in acute ischemic stroke – current status

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Abstract

With the growing understanding of the mechanism of cell death in ischemia, new approaches for treatment such as neuroprotection have emerged. The basic aim of this strategy is to interfere with the events of the ischemic cascade, blocking the pathological processes and preventing the death of nerve cells in the ischemic penumebra. This concept involves inhibition of the pathological molecular events which eventually leads to the influx of calcium, activation of free radicals and neuronal death. Despite encouraging data from experimental animal models, all clinical trials of neuroprotective therapies have to date been unsuccessful. This article reviews some of the reasons for the failure of neuroprotection in the clinical trials so far. Despite all the negative reports, we believe it would be wrong to give up at this point, since there is still reasonable hope of finding an effective neuroprotection for stroke.

Keywords: stroke • neuroprotection • ischemic penumbra

Stroke is defined as a sudden neurological deficit caused by impairment in blood flow to the brain. The normal cerebral blood flow is 55 ml/100 g of brain tissue/min. [1]. During vascular ischemia there is secondary lack of oxygen and glucose, which causes changes in the intracellular metabolism and finally neuronal death. The critical level of cerebral ischemia is set at the values of 23 ml/100 g/min. In the case of rapid reperfusion up to the normal values, the functional damage is reversible. When the cerebral blood flow drops below the values of 12 ml/100 g/min. the ischemic cascade is rapidly initiated and an infarction is caused. The peri-infarct area of potentially salvageable tissue is known as the ischemic penumbra. In focal cerebral ischemia of animal models infarct size correlates with the number of peri-infarct spreading depression like depolarizations [2]. These depolarizations are generated in the border zone of the ischemic lesion and spread into the peri-infarct tissue. Rapid reperfusion to this region may salvage this tissue. This rationale stands in the basis of thrombolytic therapy in the phase of acute stroke. The metabolic changes in the ischemic cascade include increased extracellular potassium (K) levels, depletion in ATP, suspension in protein synthesis, increased intracellular calcium (Ca) levels, decreased PH, accumulation of free radicals and lactic acid, cell swallowing due to increased intracellular water content and eventually neuronal death [3, 4]. This cascade of events is mediated by several neurotransmitters especially glutamate, *i.e.* stimulation of glutamate receptors leads to further influx of calcium activating proteolytic activities resulting in cell death. Recently several studies emphasized the role of inflammation in the acute phase and the correlation between inflammation and early and late clinical outcome, early clinical worsening, and extent of brain damage [1]. The mechanisms of ischemic neuronal death have not been fully defined and the relative contribution of apoptotic 'programmed cell death' and necrotic processes remain controversial [5].

With the growing understanding of the mechanism of cell death in ischemia, new approaches for treatment, apart of reperfusion, have emerged. Clinical trials evaluating neuroprotective drugs for stroke were first initiated during the 1980s and are still in progress [6]. Although the definition of neuroprotection is not always clear, the basic aim of this strategy is to interfere with the events of the ischemic cascade by focusing on one or more of these mechanisms of damage and, blocking the pathological processes and preventing the death of vulnerable nerve cells in the ischemic penumbra [7]. This concept involves inhibition of the pathological molecular events which eventually leads to the influx of calcium, activation of free radicals and neuronal death. That excludes, per definition, reperfusion modalities or drugs aimed to reduce the vasogenic oedema surrounding the infarct. The list of neuroprotective drugs tested in phase 2 and phase 3 trials so far is tremendously long. These agents include, among others, calcium channel blockers, calcium chelators, free radicals scavengers and antioxidants, GABA antagonists, AMPA antagonists, competitive and non-competitive NMDA antagonists, Glycine site

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antagonists, polyamine site antagonists, growth factors, inflammation blockers, adhesion inhibitors, nitric oxide inhibitors, opioid antagonists, serotonin antagonists, sodium channel blockers and potassium channel blockers. Some of the drugs evaluated, such as the case of piracetam, were of uncertain mechanism [8].

Despite encouraging data from experimental animal models demonstrating large reduction in pathological infarct volume in focal and global ischemia [9], all clinical trials of neuroprotective therapies have to date been consistently unsuccessful [7, 10]. Some neuroprotective agents have shown beneficial effect only on *post hoc* analysis [8]. The only efficacious therapies so far for acute ischemic stroke have been the ones that restore perfusion. Some arguments have been suggested to explain why neuroprotection works in pre-clinical experimental models but not in human beings [6]. One possible explanation for this striking discrepancy maybe the time window for the administration of the agent [10, 11]. In many of the animal models the drug was given shortly after stroke was induced, in contrast with the clinical set up in which there is a substantially longer time window between onset of symptoms and drug administration. In some of the pre-clinical studies the drug was given even prior to the vessel occlusion. The large trials demonstrating the benefit of thrombolysis for stroke have taught us that time is one of the most cardinal determinants of outcome [12, 13]. According to experimental data, on average, 2 million nerve cells die every minute of arterial occlusion [14]. Not surprisingly the pre-clinical trials in which a very short time window prior to treatment was used had a greater chance to be efficacious since there is still a potentially salvageable tissue. One cannot rule out the clinical efficacy of some of the drugs had they been given earlier in the time course. In addition, the outcome in the animal studies was evaluated early after the induction of stroke; such end-points maybe misleading since the clinical trials used a significant longer follow-up period for assessment of outcome. Not only the time course of end-points evaluation but also the term of outcome measures is different. The pre-clinical studies used the reduction of infarct volume, demonstrated by imaging or pathology, as the primary end-point. In contrast, the human studies used clinical and functional end-points such as the ability to walk independently or to take care of everyday needs. These functional scales include, among others, the modified Rankin Scale and the Bartel Index. It should be emphasized that the standard end-point in clinical trials, functional outcome at 3 months, is influenced by numerous factors, beside efficacy of the drug, including comorbidity, intensity of physical therapy, secondary complications, social and environmental factors, etc. [15].

Another potential explanation for the failure of neuroprotective drugs in clinical trials despite their benefit in animals is that most of the experimental models used young healthy rats which were not exposed to other medications, whereas stroke patients had often other severe comorbidities and used other drugs for variety of reasons [10]. In addition, most of the pre-clinical studies used middle cerebral artery occlusion as a model for ischemic stroke and therefore do not mimic the pathophysiological heterogenicity of different stroke types included in the clinical trials, as well as their extent, duration of ischemia and severity [16, 17]. The diversity of stroke types included in the clinical trials reduced the likelihood of showing significant efficacy. Other physiological variables that were tightly controlled in animals but not in the clinical set up include glucose levels, blood pressure and temperature as well as other metabolic factors in the acute phase. These parameters were shown to have a significant impact upon outcome in prior studies [18].

Pharmacological factors for the failure of phase 2 and 3 trials should also be taken into consideration. These factors may include insufficient dose, inadequate treatment duration, slow availability of the drug at the target area and irrelevance of the agent to human ischemia. Due to adverse events many human trials failed to match the plasma levels of the drug to the pre-clinical studies, resulting in the administration of lower doses than proven in the animal model [15].

Maybe the most significant concern is that animal studies focused on the protection of the grey matter from the tissue damage caused by the ischemic cascade with uncertain relevance to glia or white matter. However, the clinical trials also included a high proportion of patients with sub-cortical strokes and diffused white matter damage [6, 10]. In addition, the rodent brain has a higher grey to white matter ratio which may put in question the appropriateness of the experimental models [19]. NMDA antagonists, for instance, influence neuronal cell body survival [8, 15] but probably have no effect on white matter injury. The same is true regarding the role of glutamate in cortical infarct but not in lacunar stroke.

Another matter that needs to be addressed is whether the phase 3 trials have been too small to have a statistical power to detect effects that maybe clinically meaningful. Samsa *et al.* [20] demonstrated that the use of data from phase 2 studies tends to lead to overestimation of the efficacy of treatment, *i.e.* the large reductions in the pathological infarcts size in animals was thought to be translated into large clinical and functional effect in human beings. This overestimation, led to a serious reduction in statistical power of phase 3 trials.

The perception that neuroprotection always works in experimental models but never in humans has put the future of neuroprotection in jeopardy. In his provocative and stimulating comment, Rother [11] summarizes that neuroprotection does not work because it is unlikely that a neuroprotective drug reaches high enough pharmacological levels to prevent progression of tissue damage in the ischemic penumbra, therefore, combination with thrombolytic treatment is mandatory. This approach that neuroprotection should be administrated only as adjunct to thromplysis should be considered seriously since most of the neuroprotective drugs focus only on a single step among many in the ischemic cascade; therefore cannot prevent cell death without reperfusion. In contrast with the previous approach, Donnan and Davis still believe that neuroprotection is achievable in human beings [21]. They favour a stepwise approach including an initial demonstration of an effect in human cell cultures using the oxygen–glucose deprivation model. This should be followed by demonstration of penetration of drug into the ischemic region using positron emission tomography and selection of patients for surrogate outcome clinical trials with penumbral selection on neuroimaging.

We believe that despite all the negative reports, it would be wrong to give up at this point, because there is still reasonable

hope of finding an effective neuroprotection for stroke. The new insights regarding stroke pathophysiology, the understanding of previous methodological errors in the different studies, taken together with the high prevalence and burden of the disease, all speak against the premature abandonment of this approach. There are still some promising phase 3 neuroprotective agents such as DP-b99, a derivative of the calcium chelator 1,2-bis(o-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid (BAPTA) (D-pharm study) [22] and the Cerebrolysin [23] which has exhibited neuroprotective as well as neurotrophic properties in various animal models and has shown clinical efficacy and good safety in several small controlled clinical studies in ischemic stroke. A large double-blind placebo-controlled randomized clinical trial was launched in Asia to prove the validity of this treatment strategy.

Whether animals are poor indicators for clinical efficacy and do not necessarily reflect a realistic portrait of the ischemic niche in human beings or whether methodological shortcomings in trials design stand behind the apparent failure of this strategy is yet to be determined. In the future we must improve the design of clinical studies on neuroprotection considering issues such as inclusion criteria, statistical power and outcome. Combination therapy, under a strict dosing regimen, should also be considered [24].

Conflict of interest

The authors confirm that there are no conflicts of interest.

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