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Clinical trials with neuroprotective drugs in acute ischaemic stroke: are we doing the right thing?

Jacques De Keyser, Geert Sulter and Paul G. Luiten

Ischaemic stroke is a leading cause of death and long-lasting disability. Several neuroprotective drugs have been developed that have the potential to limit ischaemic brain damage and improve outcome for patients. While promising results with these drugs have been achieved in animal stroke models, all Phase III trials conducted so far indicate that these drugs have failed to live up to their promise. Despite the limits of animal models, which cannot mimic the clinical situation, the disappointing results of neuroprotective trials might largely be due to methodological problems. Future trials with neuroprotective drugs should be performed in stroke (care) units, after sufficient information regarding therapeutic time window, dosage, duration of therapy and safety has been gathered from pilot studies, and a better selection of target patients has been made. Much of this information can now be obtained by techniques that visualize the penumbra, such as combined diffusion-weighted and perfusion MRI. Consideration should also be given to clinical trials with well-designed combinations of treatments.

Box 1. Neurotoxic cascade in ischaemic brain injury

<table>
<thead>
<tr>
<th>Stroke</th>
<th>Reduction of blood flow</th>
<th>Depletion of energy stores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membrane depolarization</td>
<td>Glutamate release</td>
<td>Opening of voltage-sensitive Ca^2+ channels</td>
</tr>
<tr>
<td>Failure of Ca^2+ buffering systems and pumps</td>
<td>Elevation of intracellular Ca^2+ levels</td>
<td></td>
</tr>
<tr>
<td>Activation of NMDA and AMPA receptors</td>
<td>Free-radical formation</td>
<td>Inflammation</td>
</tr>
<tr>
<td>Release of cytokines</td>
<td>Lipid peroxidation</td>
<td>Apoptosis</td>
</tr>
<tr>
<td>Irreversible cell damage</td>
<td>Cell death</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 1. Neurotoxic cascade in the ischaemic penumbra.** A complex neurotoxic cascade is triggered by a focal deficit in brain perfusion. Key events are uncontrollable neuronal depolarization, an overspill of glutamate, a build up of intracellular Ca^2+ levels, the generation of free radicals, the stimulation of several catabolic enzyme systems and the induction of inflammation.

**References**


**Notes**


NMDA-receptor antagonist that acts as an open-channel blocker, have been studied in Phase III trials. However, the trials were terminated prematurely because of an unfavourable risk–benefit ratio.  
Eliprodil is a drug that is believed to reduce the action of glutamate by binding to the polyamine site of the NMDA receptor. Results were promising in Phase II trials, but a Phase III trial was halted because sequential efficacy analysis did not demonstrate a significant difference from placebo.  
Clomethiazole is an anti-epileptic drug that causes neuronal hyperpolarization by enhancing the activity of GABA at GABAA-receptors. The rationale behind its use is that it could inhibit ischaemia-induced neuronal depolarizations and counteract the actions of glutamate. The drug protected against ischaemic cell damage.
in animal models of permanent and transient focal brain ischaemia\textsuperscript{31,32}. A large Phase III trial, involving 1350 patients, produced negative results\textsuperscript{12}. A post hoc analysis suggested a beneficial effect in patients with severe stroke (total anterior circulation syndrome), which was the reason for initiating a new trial, the North American Clomethiazole Acute Stroke Study-Ischaemia (CLASS-I). This study aims to include 1200 patients with a total anterior circulation syndrome.

### NO-pathway inhibitors and free-radical scavengers

The neuroprotective effects of lubeluzole can be explained, at least partially, by a downregulation of the NO synthase (NOS) pathway, which reduces NO-related neurotoxicity\textsuperscript{33}. In a small Phase II trial, a dose of 7.5 mg lubeluzole given within 6 h of the first symptoms, followed by 10 mg per day for five days, was associated with reduced mortality. A double-dose regimen, which yielded a plasma concentration equivalent to the levels

<table>
<thead>
<tr>
<th>Drug Class</th>
<th>Trial</th>
<th>Time window</th>
<th>Duration of treatment</th>
<th>Result</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fosphenytoin</td>
<td>Na\textsuperscript{+} -channel antagonist</td>
<td>TRUST</td>
<td>4 h</td>
<td>3 days</td>
<td>No improvement of functional outcome at 3 months</td>
</tr>
<tr>
<td>Nimodipine\textsuperscript{a}</td>
<td>Ca\textsuperscript{2+} -channel antagonist</td>
<td>American Nimodipine Study Group</td>
<td>48 h</td>
<td>21 days</td>
<td>No improvement of neurological outcome at 6 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>German-Australian Stroke Trial</td>
<td>48 h</td>
<td>21 days</td>
<td>No difference in mortality or neurological outcome at 21 days</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NIMOSIS</td>
<td>48 h</td>
<td>21 days</td>
<td>No improvement of neurological outcome at 21 days</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NEST</td>
<td>48 h</td>
<td>21 days</td>
<td>No improvement of neurological and functional outcome at 12 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>INVEST</td>
<td>24 h</td>
<td>21 days</td>
<td>Unfavourable outcome in the nimodipine groups</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VENUS</td>
<td>6 h</td>
<td>10 days</td>
<td>No improvement of functional outcome at 3 months</td>
</tr>
<tr>
<td>Flunarizine</td>
<td>Ca\textsuperscript{2+} -channel antagonist</td>
<td>FIET</td>
<td>24 h</td>
<td>4 weeks</td>
<td>No improvement of neurological and functional outcome at 6 months</td>
</tr>
<tr>
<td>SeRete (CCS 19753)</td>
<td>Competitive NMDA-receptor antagonist</td>
<td>ASSIST</td>
<td>6 h</td>
<td>bolus</td>
<td>Unfavourable risk–benefit ratio</td>
</tr>
<tr>
<td>Carerat (CNS 1102)</td>
<td>Non-competitive NMDA-receptor antagonist</td>
<td>6 h</td>
<td>4 h</td>
<td>Unfavourable risk–benefit ratio</td>
<td>Unpublished</td>
</tr>
<tr>
<td>Elpropdi</td>
<td>Polyamine-site antagonist at the NMDA receptor</td>
<td>CLASS</td>
<td>8 h</td>
<td>14 days</td>
<td>No improvement of functional outcome at 3 months</td>
</tr>
<tr>
<td>Clomethiazole</td>
<td>Enhances the effect of GABA at the GABA\textsubscript{A}-receptor</td>
<td>LUB-INT-9</td>
<td>6 h</td>
<td>5 days</td>
<td>No reduction in mortality at 3 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LUB-INT-5</td>
<td>6 h</td>
<td>5 days</td>
<td>No reduction in mortality at 3 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LUB-INT-13</td>
<td>6–8 h</td>
<td>5 days</td>
<td>No improvement of functional outcome at 3 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NEAT</td>
<td>6 h</td>
<td>3 days</td>
<td>No improvement of functional outcome at 3 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ENS</td>
<td>6 h</td>
<td>3 days</td>
<td>No improvement of functional outcome at 3 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ebroen</td>
<td>48 h</td>
<td>2 weeks</td>
<td>No improvement of functional outcome at 3 months</td>
</tr>
<tr>
<td></td>
<td>Ganglioside GM1</td>
<td>Natural constituent of the cell membrane</td>
<td>EST</td>
<td>5 h</td>
<td>21 days</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SASS</td>
<td>48 h</td>
<td>28 days</td>
<td>No improvement of survival, neurological and functional outcome at 3 months</td>
</tr>
<tr>
<td></td>
<td>Citicholine</td>
<td>Natural constituent of the cell membrane</td>
<td>Cicholine in Acute Ischemic Stroke</td>
<td>24 h</td>
<td>6 weeks</td>
</tr>
<tr>
<td></td>
<td>Piracetam</td>
<td>Acts at the cell membrane and elevates cAMP levels</td>
<td>PASS</td>
<td>12 h</td>
<td>12 weeks</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EAST</td>
<td>6 h</td>
<td>5 days</td>
<td>Unfavourable risk–benefit ratio</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Only trials enrolling more than 250 patients are listed.

\textsuperscript{31} & \textsuperscript{32} & \textsuperscript{33}
associated with neuroprotection in rats, was associated with increased mortality. Although this was probably caused by an imbalance of randomization that was unrelated to the drug, three large Phase III trials of lubeluzole, involving 11,377 patients, were conducted with the 7.5 mg dose. All three trials failed to demonstrate a beneficial effect of lubeluzole on the primary outcome parameter, and further clinical development has been abandoned (T. Wessel, pers. commun.).

Tirilazad is a non-glucocorticoid 21-aminosteroid lipid-peroxidation inhibitor that acts as a free-radical scavenger. In animals treated within 10–15 min of focal ischaemia this drug reduced infarct volume. However, it did not improve overall functional outcome in two large Phase III studies. Because it was suggested that the lack of efficacy might be caused by the use of a dose that was too low (6 mg/kg/day for 5 days), higher doses were tested. These trials were stopped prematurely because of safety problems and further clinical study of tirilazad in ischaemic stroke has been suspended.

The seleno–organic compound, ebselen, which has antioxidant activity through a glutathione-peroxidase-like action, was studied in Japan. This drug appeared to improve outcome at one month, but not at three months after the start of treatment. Further efficacy studies with this compound might be justified.

**Drugs that mainly act at the cell membrane**

In preclinical studies, the ganglioside, GM1, conferred protection against ischaemic and excitotoxic insults. However, two major Phase III trials produced negative results. Because of concerns regarding a possible association with the development of Guillain–Barre syndrome, GM1-ganglioside product licences have been suspended.

After a number of small inconclusive clinical trials with citicholine (cytidine-5-diphosphocholine or CDP-choline), a multicentre dose-finding study in the USA suggested a better functional outcome in stroke patients receiving 300 mg citicholine per day given orally for six weeks. However, this result could not be reproduced in a pivotal trial involving 394 patients.

Piracetam is another compound that mainly acts on cell membranes of both neurones and blood cells. A placebo-controlled multi-centre study in Europe failed to show an improved outcome at three months. Post hoc analysis suggested an improvement in neurological outcome in a subgroup of patients treated with piracetam within 6 h of the onset of stroke. A new Phase III trial, PASS-2, has recently been initiated in order to confirm these results.

**Anti-inflammatory agents**

Within hours, endothelial adhesion molecule 1 (ICAM1) levels are increased in the zone of focal cerebral ischaemia, which allows an influx of white cells into the ischaemic brain area. Cytokines released from the invaded white cells contribute to brain-tissue damage. ICAM1 antibodies reduced infarct volume in rats, only when the model included reperfusion, but not with permanent middle-artery occlusion. Enlimomab, a murine monoclonal antibody against ICAM1, has been studied in a Phase III trial. Yet again, the results in the clinical situation did not fulfill the expectations generated in the laboratory. There was even a trend for early neurological deterioration in patients receiving active treatment. A probable explanation is that the murine antigens present in the enlimomab preparation themselves provoked an inflammatory response that cancelled out any beneficial effects by raising body temperature.

**Why were the trials negative?**

**Animal models**

Because all the Phase III stroke trials with neuroprotective drugs have failed to live up to their promise, one could argue that the animal models that have been used to test these substances have no predictive value. Focal ischaemia models can be broadly categorized into two types: permanent and reversible. In patients, both types of focal ischaemia can occur. Both forms of insult can produce a potentially salvageable penumbra. In the transient-occlusion model, reperfusion injury also adds to the injury. For most of the drugs mentioned above, neuroprotective activity has been demonstrated in different types of animal models, including permanent and reperfusion models of middle-cerebral-artery occlusion. However, animal models will never mimic the clinical situation and, therefore, these models should be regarded merely as a method to screen whether a particular compound has the ability to rescue neurones in the ischaemic penumbra when administered after the insult. Although these animal models are indispensable when investigating these compounds, experiments designed to measure functional outcome three months after the ischaemic insult in a larger number of animals, as required for Phase III trials in patients, cannot be justified because of ethical, practical and economic reasons. Reasons for the lack of efficacy that might have been the cause of failure are always speculative.

**Heterogeneity of the stroke population**

Animal data are usually collected in healthy laboratory rats of the same age, in which a standardized amount of focal cerebral ischaemia is induced by a reproducible intervention. In contrast, aetiology, location and severity of ischaemic stroke in patients is very heterogeneous. Young and elderly patients are grouped together. It is well known that elderly patients tend to have a worse outcome than younger patients because they have comorbidities that heavily affect outcome. Some patients have a large cortical infarction, whereas others have a lacunar infarction with a completely different prognosis. Some patients have a poor collateral circulation and, hence, a smaller penumbra or no penumbra at all. Some patients show spontaneous reperfusion in the early stages after stroke and tend to have a better clinical outcome than those without reperfusion. It is also hardly surprising that no benefit can be demonstrated when an operation designed to correct a particular pathophysiological disturbance is performed in a group of patients, many of whom do not have that disturbance. For example, one in four patients enrolled in a trial with clostridiole had lacunar white-matter infarctions, where there are no neuronal GABA receptors to be stimulated.

**Other factors that might aggravate brain damage**

In animal studies, other variables might affect infarct size and outcome, such as blood pressure, body temperature and oxygenation, which are all carefully controlled during the experiments. It is known that a reduction in blood pressure, hyperglycaemia, hypoxia and increased body temperature can all aggravate cerebral
More attention should be paid to properly conducted Phase III stroke trials conducted so far. Therapeutic dos and adverse effects.

Doses of neuroprotective drugs that limit infarct size in animals are usually associated with adverse effects that can limit tolerable doses and prohibit their clinical use. Psychomimetic side-effects were the main reason for the premature termination of trials with NMDA-receptor antagonists. Some side-effects clearly override the putative beneficial effect of a neuroprotective drug. Examples are the detrimental haemodynamic consequences of intravenous nimodipine and an inflammatory reaction associated with the administration of enlimomab. Normally, such problems should be detected in properly conducted Phase II trials, but there is often so much pressure from senior management in pharmaceutical companies to rush for registration that well-conducted Phase II trials are often neglected.

In some trials, suboptimal doses are used because too much emphasis is placed on safety aspects, although side-effects might be acceptable or properly controlled in an acute care setting. This could account for the failure of labetolol, where a possible misinterpretation of limited Phase II data and concerns about CFC-interval prolongation on the ECG led to the decision to use a dose regimen that was probably below its neuroprotective threshold. Another problem is that side-effects can limit the duration of treatment with a neuroprotective drug. Although it is not known exactly how long neuroprotective therapies should last, fear of side-effects, such as sedation, can shorten the duration of treatment to levels that are insufficient for protecting the penumbra. For example, clomethiazole was administered for 24 h (Ref. 12), although it had been demonstrated that excitatory-amine-acid levels in the ischaemic area could remain greatly elevated for at least six days after the onset of stroke.

Therapeutic time window

In many animal studies some drugs are effective only if given before or very early (between 15 min and 2 h) after the insult. Typical examples are nimodipine, tirilazad and NMDA-receptor antagonists. Other compounds, such as labetolol, are still effective in reducing ischaemic brain damage when given up to 6 h after the onset of ischaemia.

Although the penumbra in humans can exist for a longer period than in rodents, the animal experiments indicate that treatments should be started within the first few hours in order to have any chance of success. Neuroprotective drugs should be administered as long as the ischaemic cascade occurs, which can be as long as six days. Thus, optimal standard care is a prerequisite for the success of a stroke trial, and, therefore, pivotal trials with neuroprotective drugs should be performed in stroke (cerebral ounits). In addition, we should abandon the unrealistic idea that a pharmacological intervention in stroke should be applicable to all stroke types. Neuroprotective trials should be conducted in patients who are likely to have the pathophysiological disturbance that the compound was designed to treat. By using combined diffusion-weighted and perfusion MRI we should be able to identify more rationally appropriate candidates for neuroprotective therapies.

Combination therapy

All neuroprotective agents studied so far target a specific pathway of the ischaemic cascade. It is evident that the administration of either an NMDA-receptor antagonist or a voltage-dependent Ca²⁺-channel blocker will not be able to control excessive neuronal Ca²⁺ accumulation completely. Although these compounds can reduce infarct size in animal models, we should not expect that any single drug that interferes with a specific event in the ischaemic cascade will have a large clinical impact. In fact, the effects might not be measurable with the crude clinical outcome measures that are currently used, such as the Modified Rankin Scale, the Glasgow Outcome Scale or the Barthel Index. Instead of continuing with single drug trials, it might be more rewarding to explore treatments using a combination of repertusion with neuroprotection and a cocktail of carefully selected neuroprotective drugs. Animal studies have shown that combination therapies have synergistic effects. Examples are the combination of labetolol and diazepin-crosslinked haemoglobin; thrombolysis with recombinant tissue plasminogen activator (r-tPA) and a glutamate-receptor antagonist and the combination of a glutamate-receptor antagonist (MK801) with basic fibroblast growth factor.
nimbodipine, a GABA-receptor agonist,69,70 tirilazad mesylate, or citicholine.68 However, this approach would mean that pharmaceutical companies would have to work together instead of competing with each other, and that the authorities have to agree to conduct trials with compounds that have not shown efficacy on their own, with the exception of the thrombolytic drug, r-tPA, which is beneficial in a small number of ischaemic stroke patients.69,70

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LETTERS TO THE EDITOR

Cholinergic correlates of consciousness: from mind to molecules

In their recent article, Baier Perry and her co-authors illustrate elegantly that ACh is one of the important neurotransmitters that regulate consciousness. One line of reasoning is that many anesthetics appear to operate through an ACh-mediated mechanism. This proposal is based on the idea that the literature that deals with ACh. So does a possible answer lie outside the literature on ACh? Stuart Hämmerl has proposed a mechanism for anesthesia that is independent of a particular neurotransmitter. His proposal is that anesthetic gas molecules inhibit quantum states produced by endogenous van der Waals dispersion forces, which occur