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Chapter 1

Disturbances of Calcium Homeostasis in Ischaemic Stroke: Therapeutic Implications

J. Horn*, P.J.A.M. Brouwers*, M. Limburg**
*Medisch Spectrum Twente, Dept. of Neurology, Enschede, The Netherlands
** Academic Medical Center, Dept. of Clinical Informatics, Amsterdam, The Netherlands

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Abstract

In Western societies, stroke represents the third largest cause of death and the main cause of disability. With an expected increase of stroke incidence in the near future, much research is being devoted to the development of an effective treatment. At present, however, no such treatment is available, although thrombolysis may be beneficial in a small percentage of patients with ischaemic stroke.

The use of agents that protect neurones against the effects of ischaemia is appealing. Some neuroprotective drugs are believed to exert their effects by influencing calcium homeostasis in potentially viable brain cells in the ischaemic penumbra, the area surrounding the core of the infarct. A massive calcium ion ($\text{Ca}^{2+}$) influx into these cells plays an important role in the final common pathway of cell death. $\text{Ca}^{2+}$ can enter cell by voltage-sensitive calcium channels or by agonist-operated calcium channels. Calcium antagonists acting on several subtypes of these channels are capable of decreasing $\text{Ca}^{2+}$ influx into ischaemic brain cells.

In animal studies, many calcium antagonists reduce infarct size or increase cerebral blood flow. However, clinical trials have been disappointing and at present an effective neuroprotective agent has not been identified. Recently, concerns have arisen about the adverse effects of calcium antagonists acting on voltage-sensitive calcium channels.
Introduction

In the few years, major efforts have been devoted to the development of effective treatments for patients with ischaemic stroke. An effective treatment would be welcomed, since the incidence of stroke is high and over 50% of patients die or become seriously disabled.\(^1\) Strokes represent a heavy burden for patient, their family, the healthcare system, and the economy. About 4% of all healthcare costs are stroke-related.\(^2\) The incidence of stroke is expected to rise, as the number of older persons, and the average life span in industrialised countries increases ("the aging society").

Although irreversible damage to the brain occurs rapidly after arterial occlusion, in the immediate vicinity of the infarction core non-functioning brain tissue may survive for several hours. This region is called the ischaemic penumbra.\(^3\) In general there are two therapeutic strategies to minimise damage to the ischaemic penumbra: thrombolysis, to re-open an occluded artery, and neuroprotection, to protect brain tissue against the late effects of ischaemia. Thrombolysis with recombinant tissue plasminogen activator is a potentially effective treatment. However, it can be applied only to a small number of ischaemic stroke patients, in view of contra-indications and the necessity to start treatment within 3 hours after stroke onset. In this same period, brain imaging has to be performed to exclude cerebral haemorrhage or large infarction. Furthermore, treatment may be followed by serious haemorrhagic complications, including intracranial haemorrhage.\(^4,6\) Other treatment options include the use of aspirin, reducing early and late recurrences, and possibly anticoagulation, although anticoagulant therapy with unfractionated or with low molecular-weight heparin has not demonstrated unequivocal efficacy.\(^7,8\)

Neuroprotection, aimed at preservation of potentially viable cells in the ischaemic penumbra, may represent a more useful option. In animal experiments efficacy has been demonstrated for many neuroprotective drugs. Most agents could in principle be administered safely to patients with intracranial haemorrhage. Such a safety profile would allow pre-hospital treatment of stroke patients, leading to a substantial increase of the number of stroke patients that can be treated early.

This review concentrates on the therapeutic implications of clinical data from trials in stroke of agents influencing calcium metabolism. In fact, all neuroprotective agents can be considered to exert calcium antagonism, since calcium influx is the final common pathway in cell death. However, we do not discuss all potentially neuroprotective interventions, but focus on agents specifically interfering with calcium influx across the cell membrane. We briefly describe calcium metabolism in normal circumstances and in ischaemia. Different methods, advantages, and problems in animal models are discussed. The results of clinical trials of these agents are reviewed. Finally, recently reported adverse effects of calcium antagonists acting on voltage sensitive calcium channels are addressed.
**Calcium homeostasis in neurones**

Figure 1 summarises calcium metabolism in a brain cell both normally and during ischaemia.

**Under normal conditions**

Calcium ions (Ca\(^{++}\)) function as membrane stabilisers, metabolic regulators, and second messengers. They can activate intracellular degradative enzymes such as lipases, proteases, and endonucleases.\(^9\) Under normal conditions, the extracellular Ca\(^{++}\) concentration is 10,000 times higher than the intracellular Ca\(^{++}\) concentration. This large concentration gradient is maintained by active, energy-requiring mechanisms.\(^3\) Regulation takes place by precise control of membrane permeability for Ca\(^{++}\) and by extrusion of Ca\(^{++}\) from the cell by exchange (Ca\(^{++}\)-ATPase and 3Na\(^{+}\)/Ca\(^{++}\)exchange). Some intracellular Ca\(^{++}\)is bound to calmodulin or other specific binding proteins, capable of binding H\(^{+}\) or Ca\(^{++}\).\(^1\) Most intracellular Ca\(^{++}\) is sequestered in mitochondria or endoplasmatic reticulum.\(^1\) Ca\(^{++}\)can enter the cell via Voltage Sensitive Calcium Channels (VSCCs) or Agonist Operated Calcium Channels (AOCCs). L (‘long lasting’ or ‘slow’), T (‘transient’), and N (‘neither’ L or T) type VSCCs have been identified.\(^1\) They are activated (= opened for Ca\(^{++}\)) by membrane depolarisation. The main source of intracellular increase in Ca\(^{++}\) concentration is the AOCCs, which are activated by excitatory amino acids, such as glutamate and aspartate.\(^3\) Five types of AOCCs have been identified, named after the agonist that most effectively activates the receptor: high- and low affinity kainate, amino-3-hydroxy-5-methyl-4-isooazole propionic acid (AMPA), N-methyl-D-aspartate (NMDA), and quisqualate. Glutamate can activate AMPA and NMDA channels, leading to Na\(^{+}\) entering the cells through AMPA channels. This sets off membrane depolarisation. During depolarisation, magnesium ions (Mg\(^{++}\)) which normally block the NMDA channel, are removed, allowing Ca\(^{++}\)to enter. In the activation of NMDA channels two other binding sites seem to play an important role: the polyamine site.
and the glycine site. Activation of these sites is required for glutamate to open the NMDA channel. VSCCs also open during depolarisation and the influx of Ca\(^{++}\) rises further. Thus, depolarisation results in a temporary rise in intracellular Ca\(^{++}\) concentration. By re-uptake of the excitatory neurotransmitter, post-synaptic stimulation is terminated and depolarisation of the cell membrane is stopped.\(^{13}\) Potassium and chloride ion gradients are restored by extrusion of Ca\(^{++}\) and Na\(^{+}\). These processes require energy, i.e. ATP.

**In ischaemic neurons**

When cerebral blood flow (CBF) drops to 50% of normal values, electrophysiological activity disappears, thereby saving energy.\(^{14}\) In this way membrane ion gradients can be preserved and cells remain viable for at least several hours. A further decrease of CBF to ≤15 ml/100g per minute (<30% of normal), seriously affects the ability of the cell to maintain integrity. Depending on the duration and intensity of ischaemia, irreversible cell damage will ensue. Aerobic metabolism in the mitochondria is reduced and replaced by anaerobic processes, resulting in lactate production, and consequently intracellular acidosis.\(^{3}\) Binding of H\(^{+}\) to intracellular proteins in exchange for Ca\(^{++}\) is the first buffering capacity.\(^{10}\) Because of energy shortage the ATP dependent Na\(^{+}/K^{+}\) pump fails, and K\(^{+}\) leaks out of the cell. This sets off membrane depolarisation, glutamate release, opening of the AOCCs and VSCCs, resulting in an increase of Ca\(^{++}\) concentration. During prolonged ischaemia intracellular Ca\(^{++}\) concentration increases through several mechanisms: 1) membrane permeability for Ca\(^{++}\) rises; 2) the ATP dependent pump systems fail to extrude Ca\(^{++}\) from the cell; and 3) repeated anoxic membrane depolarisation increases intracellular Ca\(^{++}\).\(^{3}\) Mitochondria store Ca\(^{++}\), until they become overloaded, which causes them to swell. Permeability of the mitochondrial membrane subsequently increases, and various compounds, among which Ca\(^{++}\) and Mg\(^{++}\), are released from the mitochondrion. As a result, production of ATP stops completely, and excessive amounts of oxygen radicals, such as O\(_2\), H\(_2\)O\(_2\) and OH, are formed.\(^{15}\) This cascade of deleterious events finally leads to cell death.\(^{13}\)

In the core of the ischaemic zone these processes occur very rapidly, leading to cell death within minutes. Surrounding this core, oxygen and glucose supply is partially preserved through collateral vessels and diffusion. This so-called ischaemic penumbra is a region in which ischaemia is less dense and which contains electrically inexcitable, but essentially viable, cells for probably many hours after stroke onset.\(^{14}\) For the human brain, the exact interval for which the ischaemic penumbra remains viable is unknown. PET scanning techniques have visualised viable tissue (in which the cerebral metabolic rate of oxygen is >1.40 ml of oxygen/100 g per minute) for up to 17 hours after stroke onset.\(^{16}\) The concept of a viable ischaemic penumbra opens up possibilities for therapeutic intervention in stroke, and has led to widespread efforts to develop agents that could reduce infarct size through blockage of pathological Ca\(^{++}\) influx.
Chapter 1

Animal models

A complete review of all available data from animal experiments is outside our scope, as this review focuses on clinical aspects of calcium antagonists. We discuss the most relevant techniques used in animal models, and some contradictory results.

Two major animal models of stroke have been used: 1) global ischaemia, comparable to a situation of lowered cardiac output in humans (e.g. cardiac arrest); and 2) focal ischaemia, similar to the occlusion of a cerebral artery. Transient global ischaemia is achieved by inducing a short period of cardiac arrest, or by temporary bilateral occlusion of the carotid arteries (the species used in this model have an incomplete circle of Willis, thus causing global forebrain ischaemia), sometimes combined with systemic hypotension. Focal ischaemia is induced by ‘open’ or ‘closed’ occlusion of a middle cerebral artery (MCA). ‘Open’ refers to occlusion of the MCA by applying a clip or ligature during surgery or by inducing ischaemia by means of photochemical injury to cortical vessels. In ‘closed’ procedures brain infarctions are induced by placing a filament into the lumen of the MCA. The results of various animal studies are difficult to compare as experimental conditions, for example method of anaesthesia, temperature during ischaemia, species, mode of inducing ischaemia, route of drug administration, duration of ischaemia and reperfusion, and method of measuring the result (histopathological or ‘functional’ assessment) vary widely. Also, infarct size is highly variable in the same species, even when using similar techniques.

To exemplify the animal studies, we will briefly discuss the results with nimodipine. This 1,4-dihydropyridine supposedly has neuroprotective properties by decreasing Ca$$^{++}$$ influx through L-type VSCCs. Nimodipine was one of the first agents investigated for this indication, and the results of the first animal studies were reported in 1982.

In the global ischaemia model, nimodipine was tested in different species when administered both before and after induction of ischaemia. The duration of ischaemia was variable. An increase in CBF was reported, not always leading to improvement of neurological status. More studies have been performed using the focal ischaemia model, mainly in rats. Medication was administered before or after occlusion of the MCA, and different outcome measurements were used. The first 3 studies did not show any change in infarct size and reported different effects of nimodipine on CBF; Sauter and Rudin were the first to report on neurological outcome, which was blindly assessed by a simple neurological score judging posture, movement, and paralysis of the hind paws. If treatment was started before occlusion, improvement was found; treatment after ischaemia did not show any effect. Some later studies reported positive effects of nimodipine, others were negative. Based on the available positive results, studies with nimodipine were initiated in humans.
After nimodipine many other agents were tested in animal models, and new studies continue to be published. An overview of available animal studies appeared recently. Not all substances with encouraging results in animal studies have been tested in properly conducted randomised controlled clinical trials.

The danger of extrapolating the positive results of animal studies to the human situation, was recently pointed out by Grotta. In animals, experimental conditions such as brain temperature and blood glucose levels, which seem to have an effect on outcome in humans after ischaemic stroke, can be controlled more strictly. Compared with the adolescent animals used in most experiments, humans with ischaemic strokes are relatively older. Finally, in animal studies treatment will start immediately or within several minutes after induction of brain ischaemia. Sometimes the drug was administered even before onset of ischaemia. In clinical treatment of stroke therapy, it will always take considerably more time before neuroprotective drugs can be administered.

**Clinical trials with calcium antagonists in ischaemic stroke.**

*Agents acting on Voltage Sensitive Calcium Channel*

**Nimodipine**

The first clinical results of nimodipine in patients with stroke were reported in 1984. In a single blind study, 29 patients were treated with oral nimodipine 40mg 3 times daily for 28 days. Outcome was measured with the Mathew impairment scale. Patients in the control group (31), received the 'standard regimen' (10% depolymerized dextran for 12 hours per day during a period of 15 days). The method of randomisation was not described and no placebo medication was used. Neither was it described who was blinded to treatment arm: the physician who assessed clinical outcome or the patient. In the treatment group, 15 patients had a good outcome, 10 fair, 2 poor, and 2 died. In the control group, 10 patients were graded as having a good outcome, 7 as fair, 8 as poor, and 5 died (1 patient was not accounted for in the article). These results prompted a randomised controlled clinical trial with oral nimodipine, 30mg 4 times daily, which was published in 1988. A total of 186 patients was included (93 in each group) and treatment was started within 24 hours of stroke onset. Of the 93 patients treated with nimodipine, 16 died during the 6-month follow-up, compared with 27 in the placebo group. Most deaths were the result of pulmonary complications. Neurological deficit (assessed with the Mathew scale) significantly improved in the nimodipine group, compared with placebo. The subgroup of patients with a moderately severe stroke seemed to benefit most from therapy. However, the analysis was not a direct comparison of the clinical status of the patients, but included indirect transformations of the Mathew scale results. This 'positive' study sparked a number of subsequent trials with nimodipine in stroke patients. We summarise results of available published data in
Table I: Published trials with Nimodipine.

<table>
<thead>
<tr>
<th>Author / Study Name</th>
<th>No. in treatment / control group</th>
<th>Route, dosage and duration</th>
<th>Follow-up (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gelmers 1984</td>
<td>29 / 31</td>
<td>oral, 30 mg q.i.d., 28 days</td>
<td>4</td>
</tr>
<tr>
<td>Sherman 1986</td>
<td>11 / 11</td>
<td>oral 30 mg q.i.d., 21 days.</td>
<td>8</td>
</tr>
<tr>
<td>Gelmers 1988</td>
<td>93 / 93</td>
<td>30 mg q.i.d., 28 days</td>
<td>26</td>
</tr>
<tr>
<td>Paci 1989</td>
<td>19 / 22</td>
<td>40 mg t.i.d., 28 days</td>
<td>4</td>
</tr>
<tr>
<td>Bogousslavsky 1990</td>
<td>30 / 30</td>
<td>30 mg q.i.d., 14 days</td>
<td>16</td>
</tr>
<tr>
<td>Heiss 1990</td>
<td>14 / 13</td>
<td>i.v., 1mg/hr 2 hrs, 2mg/hr 5 days, oral 30 mg q.i.d. 16 days.</td>
<td>26</td>
</tr>
<tr>
<td>INWEST 1990</td>
<td>195 / 100</td>
<td>i.v., 1 mg, or 2 mg/hr 5 days, oral 120 mg/day 16 days</td>
<td>24</td>
</tr>
<tr>
<td>Martinez-Vila 1990</td>
<td>81 / 83</td>
<td>30 mg q.i.d., 28 days</td>
<td>4</td>
</tr>
<tr>
<td>TRUST 1990</td>
<td>607 / 608</td>
<td>40 mg t.i.d., 21 days</td>
<td>24</td>
</tr>
<tr>
<td>Bridgers 1991</td>
<td>138 / 66</td>
<td>i.v. 1 mg or 2 mg/hr 5 days, oral 120 mg/day 17 days.</td>
<td>3</td>
</tr>
<tr>
<td>Mohr 1992</td>
<td>800 / 264</td>
<td>oral, 20 mg or 40 mg or 80 mg t.i.d., 14 days</td>
<td>3</td>
</tr>
<tr>
<td>CANWIN 1993</td>
<td>96 / 93</td>
<td>i.v., 2mg/hr 10 days, oral 180mg/day 6 months.</td>
<td>52</td>
</tr>
<tr>
<td>NEST 1993</td>
<td>437 / 443</td>
<td>30 mg q.i.d., 21 days</td>
<td>12</td>
</tr>
<tr>
<td>German-Austrian 1994</td>
<td>239 / 243</td>
<td>30 mg q.i.d., 21 days</td>
<td>26</td>
</tr>
<tr>
<td>Kaste 1994</td>
<td>176 / 174</td>
<td>30 mg q.i.d., 21 days</td>
<td>52</td>
</tr>
<tr>
<td>Wimalaratna 1994</td>
<td>146 / 69</td>
<td>120 mg or 240 mg/day, 16 weeks</td>
<td>24</td>
</tr>
</tbody>
</table>

Table I. None of the published studies reproduced the results from the initial trial. The INWEST trial, in which nimodipine was administered intravenously, was terminated early, because of an unfavourable outcome in the 2 treatment groups compared with placebo. Nimodipine was administered intravenously at 2 different dosages (1 or 2mg/hour) for the first 5 days, followed by oral treatment with 30mg 4 times daily for 16 days. In total, 295 patients were randomised: 100 received placebo, 101 received nimodipine 1 mg/hour, and 94 received nimodipine 2 mg/hour. Although there were no statistically significant differences in mortality between the groups (33 deaths (33%) in the placebo group, 41 (40.6%) in the 1mg group, and 42 (44.7%) in the 2mg group) functional outcome, assessed with the Barthel ADL Index, was significantly worse in the
Disturbances of Calcium Homeostasis in Ischaemic Stroke

2mg group compared with both the 1mg group and the placebo group. Functional outcome in the 1mg group was also significantly worse compared with placebo recipients. Thus, this unfavourable result seemed to be dosage-dependent and was correlated with a reduction of mean blood pressure in both active treatment groups (see below). Two further trials with oral nimodipine 40mg 3 times daily in ischaemic stroke, each including over 1000 patients,\textsuperscript{45,46} did not show any significant effect of active treatment. Both trials included patients up to 48 hours after stroke onset. In the American Nimodipine Study Group trial,\textsuperscript{46} a subgroup analysis of patients receiving trial medication within 18 hours after stroke onset, suggested a beneficial effect of treatment with nimodipine. The investigators stated that time between stroke onset and start of treatment might be a crucial factor. In 1994, 2 meta-analyses of all available trials with nimodipine in acute ischaemic stroke were published.\textsuperscript{47,48} The study of Mohr et al, comprised 9 trials including 4324 patients, was restricted to oral medication at a dosage of 120 mg/day and allowed patient inclusion up to 48 hours after stroke onset. Neurological impairment and functional status were used as outcomes. A time-dependent effect was reported. For treatment started within 12 hours after stroke onset, a favourable effect of nimodipine was suggested for neurological impairment (odds ratio 0.62%, 95% C.I. 0.44-0.87) and functional outcome (not quantified). Patients randomised to nimodipine between 12 and 24 hours after stroke onset had no improved outcome compared with placebo. Treatment started more than 24 hours after stroke onset was associated with deterioration (Figure 2). The meta-analysis by Di Mascio et al demonstrated that nimodipine was effective in patients with subarachnoid haemorrhage but not in those with stroke. However, no analysis on time between stroke and start of treatment was performed.

Based on the positive results reported by Mohr, the placebo-controlled VENUS trial was started in the Netherlands. In this trial stroke patients are randomised by family physicians to receive nimodipine (30mg 4 times daily within 6 hours after stroke onset) or placebo.\textsuperscript{49} The

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nimodipine</td>
</tr>
<tr>
<td>All</td>
<td>1810</td>
</tr>
<tr>
<td>Male</td>
<td>954</td>
</tr>
<tr>
<td>Female</td>
<td>854</td>
</tr>
<tr>
<td>Age (y) ≤ 65</td>
<td>605</td>
</tr>
<tr>
<td>Age (y) &gt; 65</td>
<td>1202</td>
</tr>
<tr>
<td>Heart disease</td>
<td>681</td>
</tr>
<tr>
<td>Hypertension</td>
<td>783</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>299</td>
</tr>
<tr>
<td>BP &gt; 160/95 mmHg</td>
<td>717</td>
</tr>
<tr>
<td>Mild deficit</td>
<td>542</td>
</tr>
<tr>
<td>Moderate/severe deficit</td>
<td>1267</td>
</tr>
<tr>
<td>Start ≤ 12h</td>
<td>330</td>
</tr>
<tr>
<td>Start 13-24h</td>
<td>450</td>
</tr>
<tr>
<td>Start &gt; 24h</td>
<td>803</td>
</tr>
</tbody>
</table>

Fig. 2. Results of a meta-analysis of functional outcomes of treatment with oral nimodipine in ischaemic stroke (reproduced from Mohr et al., with permission). 'Start' is delay to start of treatment after stroke. \textit{BP} = blood pressure.
results of this trial indicated that early treatment with nimodipine was not effective in patients with stroke.

**Flunarizine**

A beneficial effect of flunarizine was suggested by a small pilot study in 26 patients. Treatment was begun within 24 hours of stroke onset, with intravenous flunarizine for 24 hours, followed by 11 days of oral flunarizine. At 6 months after stroke onset, outcome was assessed with the Barthel ADL Index and the Rankin scale. A favourable outcome on the Rankin scale was 32% more likely to occur in the flunarizine group (not statistically significant), compared with placebo. Two larger trials with flunarizine followed (FIST, with 331 patients treated within 24 hours, and the German flunarizine trial of 433 patients treated within 36 hours). The FIST trial was terminated prematurely because no effect was found during interim analyses and it was ‘regarded highly unlikely that a beneficial effect would appear if more patients were included’. Subgroup analysis on patients treated within 6 hours after stroke onset suggested a decreased risk for death or disability in flunarizine-treated patients (RR 0.77, 95% C.I. 0.55-0.88). The results of the German flunarizine trial were reported somewhat enigmatically. In 1 of the 5 participating centres an inhomogeneity between groups was found with regard to sensorimotor functions subscore, and these data were deleted. In the final publication no significant effect of treatment with flunarizine was reported.

**Nicardipine and others**

A controlled double-blind trial of oral nicardipine in patients with focal cerebral ischaemia was conducted in Spain. Patients were randomised to receive nicardipine 60 mg/day (n = 27) or placebo (n = 24) for a maximum of 30 days. Outcome was assessed using a 3-level scale (‘unfavourable’, ‘unchanged’ or ‘favourable’) and a functional score. No significant beneficial effects were associated with nicardipine treatment. Another trial with nicardipine and a trial with isradipine remained unpublished so far (personal information Orgogozo, France).

**Agents acting on Agonist Operated Calcium Channels**

**NMDA antagonists, noncompetitive and competitive.**

Agents acting postsynaptically on NMDA channels have been investigated thoroughly. Two types can be distinguished: noncompetitive and competitive NMDA antagonists. Noncompetitive NMDA antagonists need an already open NMDA channel, without the Mg$^{++}$ blockage, to effectively prevent Ca$^{++}$ influx. This situation exists in regions with a high glutamate concentration (i.e. ischaemia), and therefore noncompetitive NMDA antagonists are thought to accumulate in these areas. High-affinity noncompetitive NMDA antagonists bind strongly to the open channels
and dissociate slowly. This mechanism increases the number of blocked NMDA channels for prolonged periods, which may increase the intensity of adverse effects often seen with these agents. Binding of low-affinity noncompetitive NMDA antagonists is less strong, thereby minimising their effect on physiological glutamate transmissions.

The development of several drugs that were proven effective in animal studies was stopped because of serious adverse effects. For example, dizocilpine (MK-801), a very effective high-affinity noncompetitive NMDA antagonist, was never tested in clinical setting, probably because of concerns about vacuolation in neurons in animal models.\(^5\)

Dextrophan was studied in a placebo controlled, multicentre ascending dose phase II trial.\(^5\)
Stroke patients were treated within 48 hours after stroke onset and received 475 to 1280mg in 12 hours or 945 to 2140mg in 24 hours. CNS adverse effects (nystagmus, somnolence, agitation, hallucinations, confusion, and nausea or vomiting) were seen in many patients. Transient, but significant and clinically concerning, hypotension was seen in many patients. Dextrophan, the parent compound of which, dextromethorphan, is widely used at lower dosages as an anti-tussive, was not developed further for stroke treatment.

Aptiganel (CNS 1102 or Cerestat) was tested in healthy volunteers and in stroke patients.\(^5\) Dose-dependent adverse events (hypertension and profound sedation) were reported in stroke patients in dosages \(^3\) 45\(\mu\)g/kg. A randomised placebo-controlled phase III trial of aptiganel was initiated.\(^6\) The aim was to include 900 patients in the 2 treatment arms (3mg bolus injection followed by 6mg over 12 hours, or 5mg bolus followed by 9mg over 12 hours starting within six hours after stroke onset). Outcome was to be measured by the modified Rankin scale at 3 months after stroke. The trial has been stopped, the results remain unpublished.

Remacemide hydrochloride, originally developed as an anticonvulsant was tested in a placebo-controlled phase II trial.\(^6\) Stroke patients received up to 600mg twice daily (initially intravenously, oral continuation) for 6 days. The most common treatment attributed adverse events were CNS or psychiatric effects of treatment. These were observed in the two highest dose groups (500mg and 600mg), presumably due to accumulation. A dose higher than 200mg twice daily yielded "neuroprotective" plasma concentrations.

Selfotel, a competitive NMDA antagonist, had been tested in phase II and phase III trials. In a phase II trial (4 treatment groups (n = 6) and one small (n = 2) placebo group), stroke patients were treated within 12 hours.\(^6\) Dose related adverse CNS events (hallucinations, agitation, and confusion) were common. Some patients needed supportive treatment. Based on these results 1.5mg/kg was concluded to be well tolerated. The phase III trial was terminated early because mortality from brain related events was 13% in the treatment group, compared with 5% in the placebo group.\(^6\) No effect of Selfotel was found in the primary outcome analyses. Statistically higher proportions of patients in the Selfotel group suffered from agitation, hallucinations or confusion.
Mg$^{++}$ act as endogenous noncompetitive NMDA channel blocker and has neuroprotective capacities, demonstrated in animal models. Results from a randomised double-blind placebo controlled pilot trial of intravenously administered magnesium sulfate were reported by Muir and Lees. Stroke patients were treated within 12 hours after stroke onset. No serious adverse CNS events were found, nor were any cardiovascular effects observed. Outcome, as measured with the Barthel ADL Index, was not significantly different between the treatment groups. A larger randomised double-blind placebo-controlled trial of magnesium in stroke (IMAGES) is ongoing.

**Glycine- and Polyamine site antagonists**

Agents directed at the glycine and polyamine sites have been successfully tested in animal models. Eliprodil, a polyamine site antagonist, has been studied in healthy volunteers. Adverse events reported included a dose dependent prolongation of QT interval, vertigo, and drowsiness. Phase III trials with eliprodil have been conducted but were terminated early because no beneficial effect was found. Results have not yet been published in full.

The glycine site antagonist gavestinel (GV1500526) has been studied in phase II trials. Patients were treated within 12 hours after stroke onset, serious adverse events were not reported. Two large double-blind placebo-controlled trials of gavestinel were started. The results of GAIN international were recently reported, treatment within 6 hours after stroke onset did not improve outcome after stroke. The results of GAIN Americas are expected to be published soon.

Licostine or ACEA 1021, another glycine site antagonist has recently been tested in a phase II trial. In high dosage groups moderate adverse events (neurological and gastrointestinal complaints) were reported.

**AMPA channel antagonists**

Blockade of AMPA channels can prevent opening of NMDA channels. Some AMPA channel antagonists have been tested in animals. Positive results with YM-900 (YM90k) in healthy volunteers have been published. A phase II study with YM872 is ongoing in patients with acute ischaemic stroke.

**Pre-synaptic, glutamate release inhibitors.**

In animal studies, lubeluzole was reported to prevent the increase of extracellular glutamate in peri-infarct regions and diminish infarct size. In a placebo-controlled phase II trial, stroke patients were treated within 6 hours after stroke onset. Treatment consisted of an intravenous loading dose of lubeluzole 7.5mg followed by five days of 10 mg/day, or a loading dose of 15mg followed by 20 mg/day. Mortality was higher in the 20mg group, but this could be explained by
skewed randomisation with an excess of patients with initially severe neurological deficits. The 10mg group showed a decrease in mortality and the drug was well tolerated. Two phase III trials followed.\textsuperscript{87,88} Mortality was the primary end point, and secondary end points were survival time, neurological recovery, functional status (Barthel ADL Index) and level of disability (modified Rankin Scale) after 12 weeks. In the American trial\textsuperscript{87} a nonsignificant beneficial effect for mortality was found, together with a statistically significant improvement in neurological recovery. The combined end-point of death and moderate or severe disability did not show a statistically significant effect.\textsuperscript{89} In the European trial\textsuperscript{88} no significant difference for any parameter was found. All further development of lubezulole has been discontinued.

Sipatrigine (619C89) has been studied in healthy volunteers and in acute stroke patients treated within 12 hours after stroke onset.\textsuperscript{90-94} Patients receiving the high-dosage regimen reported hallucinations, but other serious adverse events were not reported. Further studies are ongoing.

### Adverse effects of calcium antagonists

As yet, a convincing benefit of calcium antagonists in the treatment of acute ischaemic stroke has not been reported and there have been suggestions of deleterious effects.

The only randomised clinical stroke trial with a calcium antagonist acting on VSCCs in stroke that has been terminated early because of an unfavourable outcome in the treatment group was the INWEST study.\textsuperscript{44} As would be expected with a calcium antagonist, intravenous treatment with nimodipine was associated with profound decreases in blood pressure. On the second day of treatment, systolic blood pressures were on average 8.3, 14.7, and 23.0 mmHg lower in the placebo group, the 1mg/hr group and the 2mg/hr group, respectively, compared with blood pressure at randomisation. Diastolic blood pressure decreased on average by 5.9, 8.8, and 15.4 mmHg, respectively. This decrease was statistically significant and concurred with poorer functional outcome. During oral treatment no significant blood pressure changes were found. These results from INWEST suggest a strong relationship between hypotension and poor outcome.

Short-acting calcium antagonists may increase mortality after myocardial infarction.\textsuperscript{95,96} The Multicenter Isradipine Diuretics Atherosclerosis Study (MIDAS) in hypertensive patients reported a statistically non-significant increase in major vascular events (stroke, myocardial infarction, sudden death, congestive heart failure, angina pectoris and other major vascular disease or death) in patients treated with isradipine.\textsuperscript{97}

Calcium antagonists may have an inhibiting effect on platelet aggregation, that might increase the risk of haemorrhage.\textsuperscript{98} In animal studies an increased bleeding time after use of calcium
antagonists was observed. A significant increase in gastrointestinal haemorrhages in hypertensive patients treated with calcium antagonists compared to beta-blockers or diuretics was reported. Legault et al. reported the early termination of a trial in patients receiving nimodipine for protection against cerebral ischaemic complications after cardiac valve replacement. Nimodipine did not protect against neurological deficits, but major bleedings were reported in 10 nimodipine-treated patients, compared with 3 in the placebo group. These results have been challenged on the grounds that significant differences existed between the two treatment groups with regard to history of pulmonary disease and congestive heart failure, influencing outcome unfavourably in the nimodipine-treated group. The use and interpretation of estimated hazard ratios, and the lack of presentation of confidence intervals were criticised. Early termination of the trial may have caused bias. Data from other groups of patients treated with nimodipine shortly before surgery, e.g. patients with a subarachnoid haemorrhage and/or trauma, do not suggest a haemorrhagic effect of nimodipine.

A variety of adverse effects have been reported with agents acting on AOCCs (see the discussion of individual drugs). In general, hypotension does not seem to be a problem, and most adverse effects concern the CNS. It is too early to conclude whether or not the use of these drugs will be limited by their adverse effects.

Conclusions

The hypothesis of an ischaemic penumbra and the elucidation of the molecular mechanisms underlying cell death, including the central role played by calcium in this process, led to many preclinical and clinical studies with drugs potentially capable of saving brain cells. Animal studies demonstrated that drugs blocking VSCCs or AOCCs could decrease infarct size after global or focal cerebral ischaemia. Randomised clinical trials followed, but no clear beneficial effect of any calcium antagonist was found. The reasons why positive animal studies may not lead to successful clinical trials have been outlined, but generally relate to more controlled environment in which the laboratory studies are performed.

Nimodipine is the most widely evaluated VSCC antagonist, but only the first clinical trial was positive. In subarachnoid haemorrhage, nimodipine has become standard therapy, following publication of the British Aneurysm Nimodipine Trial. In this placebo controlled trial nimodipine significantly reduced the number of cerebral infarcts after subarachnoid haemorrhage (34%). Patients receiving nimodipine also had a significantly better functional outcome. In subarachnoid haemorrhage, treatment starts immediately after the haemorrhage, usually before ischaemia occurs. This may offer an explanation for the effectiveness of nimodipine in this indication. Nimodipine has also been suggested to offer protection after severe head trauma. It
is possible that in these circumstances delayed cerebral ischaemia also plays a role.\textsuperscript{104}

In some stroke trials with VSCC antagonists, subgroup analyses have suggested non-significant effect of drugs in patients with moderately severe strokes or for patients treated early after stroke onset.\textsuperscript{43-46} This was also reported in a meta-analysis of nimodipine.\textsuperscript{47} However, prospective studies in which treatment is started early (within 12 hours) have yet to confirm this beneficial effect. An extensive structured review on ‘calcium antagonists for ischaemic stroke’ for the Cochrane Collaboration is in preparation.\textsuperscript{105} This study will include VSCC antagonists than nimodipine, and one of the main hypotheses to be tested is the supposedly beneficial effect of a short time interval between stroke onset and treatment. In some recent clinical trials with AOCC antagonists, treatment was started within 6 hours after stroke onset.\textsuperscript{88} Despite this short interval, no effective compound has been identified, although several trials have not yet been reported or are still ongoing.

New agents influencing calcium homeostasis in the brain are under development, and further clinical trials will extend our knowledge of their effects. A safe and effective neuroprotective agent that could be administered in a prehospital setting, would save lives and prevent many years of disability.