Understanding and managing ischemic stroke

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Abstract: Transient or permanent focal brain injury following acute thromboembolic occlusion develops from a complex cascade of pathophysiological events. The processes of excitotoxicity, peri-infarct depolarisation, inflammation, and apoptosis within the ischemic penumbra are proposed. While the translation of therapeutic agents from the animal models to human clinical trials have been disappointing, there remains an atmosphere of optimism as a result of the development of new diagnostic and therapeutic approaches, which include physiological, as opposed to pharmacological, intervention. This article provides an insight into the understanding of cerebral ischemia, together with current and future treatment strategies.

Key words: cerebral ischemia, stroke, pathophysiology.

Introduction

Acute stroke care is currently at a therapeutic threshold. On the one hand, there are the proven benefits of reperfusion therapies such as alteplase (tissue plasminogen activator) and the success of stroke units in rehabilitating patients and on the other, the failure of neuroprotective agents in clinical trials. The development of successful therapeutic strategies will result from a better understanding of the pathophysiology of focal brain ischemia (Dirnagl et al. 1999; Lee et al. 1999).

Stroke results from transient or permanent reduction in cerebral blood flow (CBF), usually secondary to thromboembolic occlusion of one or more cerebral arteries. If people reach their life expectancy, 1 in 4 men will have had a disabling stroke by age 80, and 1 in 5 women by age 85 (Wolf and D’Agostino 1986). The burden of stroke on patients, their families, and society in general is enormous. Attempts to limit brain infarct size and improve functional outcome in patients with acute stroke have focused on the restoration of blood flow and neuroprotection. There has been some groundbreaking success with the use of thrombolytic agents in acute ischemic stroke, in the context of a major clinical trial by the National Institute of Neurological Diseases and Stroke (Marler et al. 1995). However, the results of other studies that had different trial designs, although highly promising, did not reach statistical significance (Hacke et al. 1995; Hacke et al. 1998a). At the same time, there has been a complete failure of a multitude of pharmacological neuroprotective trials (De Keyser et al. 1999).

This article describes the current concepts of cerebral ischemia, which results in a complex sequence of pathological events that evolve over time and space. The current acute stroke literature is reviewed; why there are continuing controversies over the use of thrombolytic agents; and why the clinical use of neuroprotective agents might not be as straightforward as in the experimental animal model. We searched the medical literature using Medline and the Cochrane Collaboration Database, specifying subject headings for ischemic stroke, pathophysiology, and therapies.

Experimental models of ischemia: global and focal

Global ischemia

Following brief global ischemia in rodents (Pulsinelli et al. 1982) and primates (Zola-Morgan et al. 1992), delayed cell death occurs in pyramidal neurons in the CA1 sector of the hippocampus without any cell death elsewhere. The selective vulnerability of CA1 neurons appears to be related to the cell type because the same neurons are selectively injured in rodents and primates.

With prolonged global ischemia, other neurons also die with a well-defined hierarchy of susceptibility following...
ischemia of various lengths. Following 5 min of ischemia, CA1 hippocampal neurons die. Following 10 and 15 min of global ischemia in the gerbil, neurons die in the striatum, inferior colliculus, septum, and pyramidal neurons in layers 3 and 5 of the cortex, CA3 neurons of the hippocampus, and lateral, reticular, and geniculate nuclei of thalamus, and the substantia nigra.

**Focal ischemia**

Two general approaches have been used to produce focal ischemia in animals: craniotomy with vessel ligation or photoocoagulation or an extracranial endovascular occlusion utilising either a nylon suture or autologous blood clots.

Mechanisms of cell death are discussed below and their relation to global and focal ischemia emphasised. Delayed cell death that occurs in CA1 region, and possibly elsewhere in the brain, has been the subject of several interrelated theories. These include: (i) an excitotoxic hypothesis where ischemic cell death is related to excitatory amino acids at glutamate receptors and is mediated by calcium ions, (ii) a mitochondrial hypothesis where ischemia disrupts mitochondrial function, (iii) an inflammatory hypothesis where ischemia-induced changes in cells result in an inflammatory attack from leukocytes and microglia, and (iv) an apoptosis hypothesis where ischemia initiates a form of programmed cell death with the activation of killer proteins. These mechanisms, together with potential pharmacological targets, are discussed.

**The ischemic penumbra**

The penumbra is theoretically defined as ischemic brain tissue that is fundamentally salvagable (Astrup et al. 1981) and is perhaps pragmatically characterised by a response to pharmacological agents (Memezawa et al. 1992). When blood flow to the brain is reduced, survival of brain tissue depends on the intensity and duration of the ischemia and the availability of compensatory collateral blood flow. Animal experiments have allowed for estimates of thresholds of ischemia in the brain. At blood-flow levels around 20 mL·100 g–1·min –1, electroencephalographic activity is affected. The cerebral metabolic rate for oxygen (CMRO 2) also begins to fall when CBF is diminished below 20 mL·100 g –1·min –1. At levels below 10 mL·100 g–1·min –1, both the integrity of cell membranes and their electrophysiological function are severely affected (Hakim 1987). The zone of dysfunctional brain tissue surrounding the centre of the infarction (the core) has traditionally been referred to as the ischemic penumbra. Both the focal and global models of ischemia have defined the biochemical cascade that ensues following cerebral ischemia.

The extent of the brain injury is ultimately dependent on the critical thresholds of reduced CBF, the duration of the ischemic insult, the tissue temperature, the blood glucose level, and other physiological variables. During the initial few hours of vascular occlusion, different brain functions break down at widely varying CBF levels (Fig. 1). At declining flow rates in both global and focal models of ischemia, protein synthesis is first inhibited in neurons, followed by anaerobic glycolysis, the release of neurotransmitters, impaired energy metabolism, and finally membrane depolarisation (Hossman 1993). There is evidence that free radicals may also be generated in acute ischemia (Kontos et al. 1992).

**Excitotoxicity and calcium ions**

Within about 7 seconds of the onset of global ischemia,
consciousness is lost. As seconds turn into minutes, the decline in CBF and the accompanying loss of oxygen supply results in impaired energy metabolism and measurable decreases in adenosine triphosphate (ATP) and phosphocreatine. The combination of ATP breakdown and compensatory anaerobic glycolysis leads to cellular acidification. With energy depletion, the membrane potential is lost and neurons and glia depolarise (Katsura et al. 1994). This triggers Ca\(^{2+}\) influx through voltage sensitive Ca\(^{2+}\) channels which further depolarises the membrane, and excitatory amino acids are released into the extracellular space. Energy dependent processes such as glutamate re-uptake are impaired, which further increases extracellular glutamate resulting in prolonged activation of membrane glutamate receptors and Ca\(^{2+}\) influx (Siesjö et al. 1989). Sodium and chloride enter the neuron via channels for monovalent ions (Tyson et al. 1996). Water follows passively, and the ensuing edema can affect the perfusion of regions surrounding the focally injured brain, as well as having more remote effects that produce increased intracranial pressure and vascular compression.

During cerebral ischemia, the release of glutamate and the activation of glutamate receptors increases intracellular Ca\(^{2+}\) concentration, which may be a major factor in initiating cell injury (Mies et al. 1993). Several investigators have used N-methyl-D-aspartate (NMDA) antagonists in an effort to decrease ischemic brain injury. Many of these agents produce CNS toxicity and their administration may be accompanied by an undesirable systemic hypotension with associated decrease in CBF. Extracellular glycine is a co-agonist of glutamate and may also be important in the pathogenesis of ischemic brain damage. Pharmacological antagonism of a glycine site on the NMDA receptor offers a neuroprotective profile similar to that previously observed for antagonists of glutamate at the NMDA complex, with fewer side effects (Warner et al. 1995).

Glutamate has an important physiological role as a neurotransmitter in the brain. Glutamate antagonists can therefore have serious unwanted side effects, such as respiratory depression or cardiovascular dysregulation. By targeting the subunits of glutamate receptors, drugs can be developed that have more specific modes of action, making them more selective and clinically safer.

Activation of amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors following ischemia allows Na\(^{+}\) influx that contributes to the depolarisation of the neuron and to the influx of Ca\(^{2+}\) through Ca\(^{2+}\) channels. Experimental studies have suggested a greater ischemic protection with AMPA antagonists compared with NMDA antagonists (Small et al. 1999; Xue et al. 1994).

Interest in the role of metabotropic glutamate receptors in ischemia has grown recently. These do not gate ion channels but act via G proteins. There is growing evidence that activation of group II and III metabotropic glutamate receptors may be neuroprotective (Bond et al. 1998; Chiamulera et al. 1992).

Peri-infarct depolarisation

Ischemic neurons and glia depolarise because of a shortage of energy and the release of K\(^{+}\) and glutamate. In the core region of ischemic brain tissue, cells that undergo anoxic depolarisation may never repolarise. In penumbral regions, cells may repolarise but at the expense of further energy consumption. Waves of spreading depression have been demonstrated in animals occurring at a frequency of several events per hour and may be recorded for at least 6–8 hours. As the number of depolarisations increase, infarct size increases. Both NMDA and AMPA receptor antagonists block this peri-infarct depolarisation and, as a result, may decrease infarct size.

In the hours to days following ischemia, there is a disruption of Ca\(^{2+}\) homeostasis which leads to the activation of a series of Ca\(^{2+}\) dependent processes that profoundly impact the development of tissue damage, such as the activation of proteolytic enzymes that degrade both the cytoskeletal and extracellular matrix proteins (Zhao et al. 1994). Calcium activation of phospholipase A2 and cyclo-oxygenase generates free radicals (Leur et al. 1996). This, together with the increased free radical burden associated with impaired oxidative metabolism and the activation of microglia, may overwhelm intrinsic scavenging systems resulting in the peroxidation of membrane lipids (Siesjö 1992; Hall et al. 1999). Pharmacological upregulation of free radical scavenging enzymes has been shown to reduce infarct volumes (He et al. 1993).

Mitochondria

Mitochondria have been increasingly implicated in the pathophysiology of ischemic brain injury. Their function is impaired by free radical mediated disruption of the inner mitochondrial membrane and the oxidation of proteins that mediate electron transport. The mitochondrial membrane becomes leaky and eventually mitochondria become overloaded with Ca\(^{2+}\) resulting in impaired ATP production and ultimately energy and membrane failure (Budd 1998). Cytochrome C released from the mitochondria is a trigger for apoptosis (Chan et al. 1999).

Temporal and hemodynamic thresholds: a mismatch

The hemodynamic, metabolic, and ionic changes described above do not affect the ischemic territory homogeneously. In the centre or core of the perfusion deficit, cerebral blood flow is less than 10 mL·100 g\(^{-1}\) tissue·min\(^{-1}\) (Hossmann et al. 1985). Anoxic depolarisation develops minutes after the onset of ischemia. Cells are rapidly killed by lipolysis, proteolysis, the disaggregation of microtubules that follows energy failure, and the ensuing breakdown of ion homeostasis (Siesjö et al. 1998). Between the irreversibly damaged core and the normal brain lies the penumbra, an area of constrained blood flow with partially preserved energy metabolism (Astrud et al. 1981; Siesjö et al. 1998; Obrenovitch 1995). With time, the penumbra may progress to infarction because of ongoing excitotoxicity or secondary processes such as spreading depression, post-ischemic inflammation, and apoptosis.

In line with the definition above, the ischemic penumbra is a region of reduced blood supply in which the energy metabolism of the tissue is preserved. Under experimental conditions, regional blood flow and energy state can be imaged by combining \(^{14}\text{C}\) iodoantipyrine autoradiography with ATP induced bioluminescence (Hossmann et al. 1985; Mies et al. 1991). Unfortunately, this method does not allow repeated measurements. Therefore, attempts have been made to iden-
In some stroke patients, the ischemic region demonstrated to develop a concept of the ischemic penumbra in human stroke. The occlusion of the right MCA as defined by MR angiography (arrow) involving the right hemisphere (arrows). The MR, obtained within an hour of the CT, shows a hyperintensity on DW imaging resulting from occlusion of the right MCA as defined by MR angiography (arrow).

Quantitative analysis of ADC reduction in this region showed a gradual ADC decrease from the periphery to the core, the lowest ADC value amounting to about 60% of the control. A core of ATP depletion was demonstrated with an area of tissue acidosis beyond that of ATP depletion. The quantification of ADC in the ischemic territory allows the distinction between a core region (energy depleted) and an area surrounding the core with normal energy balance but severe tissue acidosis (Hoehn-Berlage et al. 1995). In rats subjected to permanent middle cerebral artery occlusion at 7 hours after the onset of ischemia, the ischemic region depicted by DW image was almost identical to the region of severe ATP decline and tissue acidosis was identified immediately after the rats were killed.

This new MR imaging technology has allowed us to develop a concept of the ischemic penumbra in human stroke. In some stroke patients, the ischemic region demonstrated by perfusion imaging is larger than the region of DW imaging abnormality during the first few hours of ischemic stroke. It has been suggested that this mismatch of ischemic territories between DWI and PWI at early time points might represent salvageable ischemic tissue, while regions of concordance may represent irreversible brain injury (Warach et al. 1995a). Animal studies using DWI revealed heterogeneous ADC values in the ischemic zone early after stroke onset that worsen and become more homogeneous over time (Reith et al. 1995). The lower the ADC, the lower is the residual CBF and the more metabolically compromised is the ischemic tissue. Animal studies are difficult to extrapolate to human stroke because in the stroke models the precise time of onset is known and the ischemic lesions are stereotyped. In human stroke, combination of DW imaging studies, ADC maps, and PW imaging are necessary to define potentially salvageable tissue (i.e., the mismatch). New tracers, for example receptor ligands or hypoxia markers, might improve the identification of this penumbral tissue. For the present, current imaging techniques can be used as surrogates to target potential therapeutic strategies for investigation in large clinical trials.

**Inflammation**

**Cytokines**

Astrocytes, microglia, leukocytes, and endothelial cells are activated by ischemia and begin to produce cytokines (Rothwell et al. 1999). Cytokines such as tumour necrosis factor α (TNFα) (Liu et al. 1994) and interleukin 1β are responsible for the accumulation of inflammatory cells in the injured brain and may affect the survival of damaged neurons. During ischemia, cytokines attract leukocytes and stimulate the production of adhesion receptors on leukocytes and endothelial cells. Leukocytes promote infarction through their toxic by-products, phagocytic action, and by the immune reaction. IL-1 receptor antagonists attenuate infarct size (Betz et al. 1995; Relton et al. 1996), while injection of IL-1β or TNFα increases infarct volume (Yamasaki et al. 1995).

**Adhesion receptors**

Cytokines induce the expression of the adhesion receptors such as intracellular adhesion molecule-1 (ICAM-1), vascular...
cell adhesion molecule-1 (VCAM-1), and endothelial cell adhesion molecule-1 (ECAM-1) (Okada et al. 1994). Adhesion receptors interact with complementary surface receptors on neutrophils. The neutrophils in turn adhere to the endothelium, cross the vascular wall, and enter the brain parenchyma (Mori et al. 1992). Macrophages and monocytes follow neutrophils and migrate into ischemic brain tissue. Modification of this post-ischemic inflammation has been shown in some laboratories to alleviate ischemic brain injury, but not in others (Takeshima et al. 1992; Toyoda et al. 1996; Bowes et al. 1993; Chen et al. 1992). Caution must be exercised in that some of the benefit of anti-inflammatory drugs in ischemia may be due to the mitigation of hypothermia because hypothermia itself also reduces inflammation. Anti-neutrophil benefits might also be accrued by an improvement in post-ischemic blood flow (Feuerstein et al. 1998; Loddick and Rothwell 1996).

**Nitric oxide inhibitors**

Production of toxic mediators by activated inflammatory cells and injured neurons has important consequences. Infiltrating neutrophils produce inducible NOS, an enzyme that produces toxic amounts of nitric oxide (NO). The pathogenic importance of NO is reflected by the observation that pharmacological inhibitors of inducible NOS reduce ischemic injury. The inflammatory process might also be linked to apoptosis because antibodies against adhesion molecules attenuate post-ischemic inflammation and reduce apoptotic cell death in the ischemic brain (Iadecola et al. 1997; Dereski et al. 1993).

**Apoptosis**

The process of apoptosis refers to ‘programmed’ cell death. Apoptosis occurs in the nervous system during development, and in part during a number of pathophysiological processes such as neurodegeneration and ischemia. It is mediated by DNA cleavage and by other autolytic processes causing nuclear shrinkage, chromatin clumping, cytoplasmic bleeding, and ultimate cell death.

Following ischemia, brain cells can die by necrosis or apoptosis (Portera-Cailliau et al. 1997a). This may depend on the nature of the stimulus, the type of cell, and the stage it has reached in its development. Necrosis is the predominant mechanism that follows acute permanent focal vascular occlusion or global ischemia, whereas in milder injury, death may resemble apoptosis. The genes for caspase, as well as the genes that suppress or augment cell death, are expressed at higher levels and activated in both the early and late stages of ischemia. Three major classes of genes control apoptosis. These include: (i) those that prevent cell death, like bcl-2 and bcl-xL; (ii) those that promote cell death, like bax and bcl-xs; and (iii) those that promote the engulfment and destruction of cells. Drugs that block caspases (Li et al. 2000) or enhance the action of bcl-2 (Adams and Cory 1998) protect against ischemic injury. Of the twelve that have been identified, caspases 1 and 3 seem to have a pivotal role in ischemia-mediated apoptosis.

To summarise, the cascade of events leading to cell death can be divided into 3 stages. The initial stage is the ischemic period during which ionic gradients collapse (60–90 minutes). The only neuroprotection strategy available during this interval is to re-establish reperfusion with thrombolysis. The second stage is the reperfusion period in which there is recovery of energy metabolism, ion homeostasis, and basic physiological functions; this stage may last hours to days. This is the interval during which neuroprotective strategies designed to prevent the death of post-ischemic neurons may be beneficial (Fig. 3). The third stage is characterised by secondary cell injury and death.

**Clinical developments: acute stroke treatment**

Hope that the traditional nihilistic approach to acute stroke care would completely change followed the publication in 1995 of the positive National Institute of Neurological Diseases and Stroke (NINDS) trial of alteplase in acute (under 3 hours) ischemic stroke. Post-hoc analysis of some of the other tPA trials (Hacke et al. 1998a, 1998b) have also indicated a benefit of the therapy over placebo for up to 6 hours after the onset of symptoms. The approval of tPA for stroke under 3 h by the US Food and Drug Administration, as well as endorsement by the American Academy of Neurology (1996), the American Heart Association, and the Canadian Stroke Consortium further increased the optimism within the medical community for the treatment of acute ischemic stroke. Nevertheless, concerns exist regarding the safety of this therapy, because of the excess risk of intracerebral hemorrhage. Another question is who is most likely to benefit from thrombolytic therapy. We will consider the evidence for stroke units, aspirin, thrombolysis, and anticoagulation.

**Stroke units**

When compared with the conventional care of stroke patients in a general medical ward environment, organized care in a stroke unit reduced death and dependency at one year after stroke with an absolute risk reduction of 5.6%. Treating 1000 acute patients in a stroke unit rather than on a general ward would prevent about 56 patients from dying or becoming dependent (Stroke Unit Trialists’ Collaboration 1999). The number needed to treat (NNT) to prevent one death was 32 (95% CI, 18 to 200), and to allow one patient to regain independence was 18 (95% CI, 11 to 45). Subgroup analysis demonstrated that the benefits were independent of age, sex, stroke severity, or design of stroke unit. Stroke units do not increase the length of stay. However, the impact of stroke units on the stroke population depends not just on how effective they are (Table 1), but also on their availability and accessibility. Rates of acute stroke admission to hospital varies, is as low as 55% in the UK and as high as 95% in Sweden (Sudlow and Warlow 1997), so the accessibility of stroke patients to acute stroke units is likely very much lower (Lindley et al. 1995). While the exact mechanism by which stroke units improve outcome is not known, it probably results from the multidisciplinary care that patients receive in these environments. Possible mechanisms include normalisation of physiological variables such as temperature, fluid and electrolyte balance, blood sugar (Sutherland et al. 1992), blood pressure, as well as rapid mobilisation, physiotherapy, and nutrition.
Aspirin

Aspirin (160–300 mg daily) given orally (or iv or rectally for those patients who cannot swallow) within 48 hours of symptom onset reduces death and dependency with no increased risk of intracerebral hemorrhage (Table 1). In absolute terms, an additional 13 patients were alive and independent per 1000 patients treated (Gubitz et al. 1999a). It is estimated that in a city with a population of 1 million, that 2000 will have an ischemic stroke per year. If they all receive aspirin within 48 hours, an additional 23 (1.2%) will be independent at one year, but this is only 1.8% of the 1300 people who die or become dependent each year after stroke (Hankey and Warlow 1999).

Thrombolysis

In Caucasians, approximately 80% of strokes are caused by occlusion of extra or intracranial cerebral arteries by thromboembolic material. The rationale for the use of thrombolytic therapy is that through rapid clot lysis, cerebral blood flow is restored, limiting the size of the infarction. Thrombolytic therapy was first evaluated in stroke patients over 40 years ago (Sussman and Fitch 1958), but its use was associated with a high incidence of fatal intracranial hemorrhage. It is only since the introduction of computerised tomography (CT) that the therapy is safe in patients with definite ischemic stroke.

The pivotal NINDS trial was the first truly positive thrombolytic study; despite an increased incidence of symptomatic intracerebral hemorrhage in the treatment group (6.4% receiving treatment versus 0.6% in controls), treatment with intravenous alteplase within 3 hours of the onset of ischemic stroke significantly improved clinical outcome at 3 months.
Table 1. The benefits, risks, treatment window, and costs for each of stroke units, aspirin, alteplase, and heparin.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Number needed to be treated to produce an independent outcome</th>
<th>Risk</th>
<th>Treatment window</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alteplase</td>
<td>6 if under 3 h; 16 if under 6 h</td>
<td>2.5–3.4 increase in odds of symptomatic intracerebral hemorrhage</td>
<td>Earlier the better!</td>
<td>$12,000 to prevent one death</td>
</tr>
<tr>
<td>Aspirin</td>
<td>83</td>
<td>One symptomatic intracerebral hemorrhage for every 500 patients treated</td>
<td>48 h</td>
<td>$83 to prevent one death or dependent</td>
</tr>
<tr>
<td>Heparin (low dose)</td>
<td>111</td>
<td>Induces 1 symptomatic intracerebral hemorrhage when 111 patients treated</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

The benefit was substantial, with 160 additional independent survivors per 1000 treated. The initial study was in fact two trials. Part 1 tested whether alteplase had clinical activity, indicated by an improvement of 4 points over baseline values of the National Institute of Health Stroke Score (NIHSS) or the resolution of the neurological deficit within 24 hours of the onset of stroke. Part 2 used a global test statistic (combining four accepted stroke scales) to assess clinical outcome at 3 months. The trial design required very early entry of a large proportion of persons. Almost half the patients were treated within 90 minutes of stroke onset. No significant differences in mortality were noted acutely or at 3 months. A significant increase in improvement at 24 hours and favourable outcomes at 3 months were noted among patients treated with alteplase. An analysis of data since the study, suggests that the earlier patients were treated, the better the outcome (Marler et al. 1999).

Three earlier trials of intravenous streptokinase in acute ischemic stroke have been performed: MAST-E, MAST-I, and ASK. All were stopped prematurely by safety committees because of increased rates of acute mortality and intracerebral bleeding (Adams et al. 1996). All compared 1.5 million U of intravenous streptokinase with a control. MAST-I also randomised patients to the combination of streptokinase and aspirin. A non-significant reduction in the likelihood of death or disability at 6 months after treatment was shown with streptokinase. However, mortality within 10 days and symptomatic intracerebral hemorrhage were significantly higher among persons who had received streptokinase. The risk of early death or bleeding was particularly high when streptokinase was combined with aspirin.

In ECASS I ( Hacke et al. 1995), patients were enrolled within 6 hours of stroke in a randomised, double blind, placebo-controlled trial of tPA. Eighty percent were treated more than 3 hours after stroke onset. Those assigned to active treatment received alteplase in dose of 1.1 mg/kg up to a total of 100 mg. The study failed to find a significant benefit in the intention-to-treat population for the primary end points (Barthel Index and modified Rankin). However, in the small percentage of patients treated under 3 hours, there was a benefit seen in patients receiving alteplase, although this time window was not sufficiently powered to show a statistically significant result. In the ECASS I study, the most frequent protocol violation was the inclusion of patients with early infarct signs of >33% of the middle cerebral artery territory. It was hoped that through better exclusion of these patients, a significant treatment effect would be seen. However, post-hoc analysis of the ECASS I data using the NINDS study statistical methodology showed a significant increase of favourable outcome in the tPA treated patient group ($P = 0.008$; OR, 1.5; 95% CI, 1.1 to 2.0) ( Hacke et al. 1998b).

In 1998, ECASS II ( Hacke et al. 1998a) was published. This trial enrolled 800 patients in Europe, Australia, and New Zealand, and treated them with alteplase 0.9 mg/kg or placebo within 6 hours of symptom onset. The investigators were successful in excluding patients with early infarct signs on CT imaging, but at the expense of including many patients with relatively mild strokes. In this study, 80% of patients were enrolled between 3–6 hours of symptom onset. No significant treatment benefit was seen in the primary end point. However, in a post hoc analysis using independent recovery (Rankin 0–2), a significant benefit was seen with 54% of the alteplase treated patients versus 46% placebo being independent.

The Alteplase Thrombolysis for Acute Non-interventional Therapy for Acute Ischemic Stroke (ATLANTIS) is the third randomised trial evaluating alteplase with the majority of patients being treated between 3–5 hours. A comparison of the ATLANTIS trial with ECASS II trial suggests that the two trial populations were quite similar. The median NIHSS was 10 in the ATLANTIS trial, compared with 11 in the ECASS II trial. The incidence of spontaneous recovery was 40% in both studies, which probably relates to the relatively mild strokes recruited and why neither study was able to show a treatment effect. However, in ATLANTIS, treatment with alteplase produced a significant increase in the percentage of patients in the target population with major neurological improvement (defined as an 11-point improvement or full recovery).

A systematic review of 17 clinical trials (Wardlaw et al. 1999) showed that thrombolysis significantly increased the
Table 2. Summary of pharmacological agents in clinical stroke trials.

<table>
<thead>
<tr>
<th>Proposed mechanism</th>
<th>Trial/name of drug</th>
<th>Result</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Calcium channel</td>
<td>Nimodipine</td>
<td>No benefit in 15 trials</td>
<td>Metanalysis suggests benefit if administered &lt;15 h</td>
</tr>
<tr>
<td>3. Na⁺ Channel</td>
<td>Lubeluzole (Grotta 1997)</td>
<td>European trial negative (Diener 1998)</td>
<td>Combined results suggest positive effect in mild to moderate strokes</td>
</tr>
<tr>
<td>4. Free radical scavengers</td>
<td>Tiriliazad</td>
<td>United States trial showed non-significant reduction in mortality a positive effect on functional outcome</td>
<td>The 3 trials are contradictory: TESS II showed an increased mortality with tirilazad. RANTASS suggested reduced mortality. RANTASS II was prematurely stopped following the results from TESS II but showed a 14% absolute reduction in mortality and a reduction in dependency (Onal and Fisher 1997; Lees 1998; Haley 1998)</td>
</tr>
<tr>
<td>5. γ-aminobutyric acid agonists</td>
<td>Chlormethiazole CLASS-H</td>
<td>No efficacy demonstrated</td>
<td>A subgroup of large strokes benefited (Wahlgren et al. 1999b)</td>
</tr>
<tr>
<td>6. Anti-intercellular adhesion molecule</td>
<td>Anti-ICAM (Enlimomas Acute Stroke Trial 1997)</td>
<td>Active drug caused fever perhaps explaining the worse outcomes</td>
<td>Worse outcome and increased mortality in the treatment group</td>
</tr>
<tr>
<td>7. Cell membrane function</td>
<td>Piracetam (De Deyn et al. 1997) Citicholine (Clark et al. 1997)</td>
<td>Not powered to show benefit. Overall no efficacy demonstrated</td>
<td>Trend towards improvement in those randomised before 7 h Improvement in functional only seen in subgroups: 500 mg subgroup; moderate to severe strokes</td>
</tr>
</tbody>
</table>
Table 3. On-going and future targets for neuroprotective agents and strategies.

<table>
<thead>
<tr>
<th>Pharmacological Action</th>
<th>Agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Nitric Oxide</td>
<td>ARL 17477</td>
</tr>
<tr>
<td>2. Cell cycle genes</td>
<td>Protooncogenes, c-fos, c-jun</td>
</tr>
<tr>
<td>3. Immediate early genes</td>
<td>Basic fibroblast growth factor, Insulin-like growth factor, Brain derived neurotrophic factor (Fisher 1998)</td>
</tr>
<tr>
<td>4. Heat shock proteins</td>
<td>(S)-BMS-204352</td>
</tr>
<tr>
<td>5. Growth factors</td>
<td>BAY 3702 (Teal 1998)</td>
</tr>
<tr>
<td>6. Specific opener of Ca** activated (Maxi-K) K+ Channels</td>
<td>Magnesium (Mg^+) (Muir and Lees 1995)</td>
</tr>
<tr>
<td>7. Serotonin agonist</td>
<td></td>
</tr>
<tr>
<td>8. Blockage of voltage dependent ion channel of the NMDA receptor complex</td>
<td></td>
</tr>
<tr>
<td>9. Hypothermia (Schwab et al. 1997)</td>
<td></td>
</tr>
</tbody>
</table>

Table 1 summarises the efficacy of an acute stroke intervention in terms of the number needed for treatment to produce an independent outcome, the risk of the therapy, the time constraints for therapy, and the estimated costs of stroke units, aspirin, thrombolysis, and heparin.

The failure of neuroprotective clinical trials

Despite numerous agents that can prevent the cascade of events leading to ischemic neuronal death in animal models (Del Zoppo 1995), there is no neuroprotective agent that has been shown to conclusively improve stroke outcome (Del Zoppo 1995; Grotta 1995; Dorman et al. 1996). The clinical trials are summarised in Table 2.

The discrepancy between animal results and clinical trials may be due to several factors: the heterogeneity of human stroke, morphological and functional differences between the brain of humans and animals, difficulty in timing when these agents may be of benefit, better experimental control of physiological variables such as temperature, blood pressure, and differences in evaluating efficacy (histology...
A drug dose effective in the mouse or rat may not be effective in large animals or humans. Pharmacokinetics and pharmacodynamics may vary considerably among species. Therefore, the generation of dose response curves is essential. Another factor to consider is the period of time after the ischemic event that drug administration may be effective. The window of therapeutic opportunity in animal models is not necessarily predictive of the time window in humans, but the determination of relative windows is useful. In animal models, the time of the stroke or ischemic onset is known precisely, whereas in humans this may not be the case. In animals, the administration of drug is at precise times: either at the onset of ischemia, immediately after reperfusion, or various times after reperfusion. In humans, pharmacological agents are administered many hours after the onset of symptoms, perhaps in the presence of a persisting occlusion. The interval after the onset of ischemia or reperfusion, when the drug can be successfully administered, should be determined to demonstrate whether the pharmacological agent can be effective at various times after the ischemic event. It is also critical that when testing a potential neuroprotective agent in animals, the experiments should be performed in a randomised and blinded fashion. Outcomes measured in animal models should include infarct volume and functional (behavioural) response. Outcome measures should be monitored during the acute phase (1 to 3 days) and the long term (7 to 30 days). Several studies have demonstrated that it is necessary to follow animals over longer periods of time because the initial benefits of agents may be lost over time. Blood pressure, haemoglobin, glucose, blood gases, and CBF should be monitored for as long as possible because variations in these parameters may be sufficient to effect outcome. One new exciting consideration concerning neuroprotective strategies involves the manipulation of endogenous enzymes that may play a role in the mechanism of ischemic injury. Neuronal nitric oxide knockout mice have smaller infarct volumes than wild-type mice (Chan 1996). In human stroke, potentially beneficial neuroprotective agents may not reach sufficient tissue concentration if the vessel supplying the territory remains occluded. It therefore seems logical that such agents, with multiple modes of action, should be administered in combination with a thrombolytic therapy. Such a strategy may lead to the development of a “stroke cocktail.”

Investigators continue to develop neuroprotective agents (Table 3). Hypothermia is of considerable interest as experimental studies have repeatedly demonstrated efficacy across species and laboratories (Bruno et al. 1994). Its application to clinical stroke, however, is complicated by the need for a general anesthetic and admission to an intensive care unit. While human trials are ongoing, the potential adverse effects of such an intervention may negate the potential beneficial effects of hypothermia in an elderly stroke population.

There are several unresolved issues regarding neuroprotection in acute cerebral ischemia. Firstly, the duration of treatment that would achieve the best effect is unknown. There is evidence that viable tissue may be present for as long as 48 hours. Secondly, the potential interaction between physiological variables and neuroprotective strategies should be considered. The four major physiological variables that must be monitored and managed are blood pressure, arterial blood gas levels, body temperature, and glycemia. The effects of these physiological variables have not been studied prospectively, although they may all contribute to the outcome of acute ischemic stroke and affect the duration of the therapeutic window. Optimal physiological parameters are considered to be neuroprotective. Trials of new pharmacological agents should aim to maintain these physiological variables as close to normal as possible.

**Conclusion**

There is now good evidence that the current management of acute stroke favours the use of aspirin, stroke units, and thrombolysis. Tissue damage following cerebral ischemia results from the interaction of complex processes such as excitotoxicity, peri-infarct depolarisation, apoptosis and inflammation. Each of these events, in the cascade of processes from cerebral ischemia to irreversible brain injury, may be a potential target for therapy. The cardinal event for cerebral ischemia is the disruption of blood flow, which in the vast majority of stroke patients is secondary to thromboembolic occlusion. Future interventions will very likely combine therapeutic strategies that enhance both reperfusion and neuroprotection.

Important issues remain unresolved regarding the translation of experimental developments to the clinical setting. Developing surrogate outcome markers such as MR will be of importance to be able to titrate the doses of new pharmacological agents, the duration of treatment, and the size of effect before launching clinical phase III trials.

**References**


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