Current Status of Neuroprotective Agents in the Treatment of Acute Ischemic Stroke

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To keep ischemic brain cells alive, neuroprotective agents target events in the ischemic cascade that might be injurious to the cells. They can be divided broadly into groups that restore ion balance, block receptors, prevent reperfusion injury, or promote neuronal healing. To date, neuroprotective agents have either shown a lack of efficacy in clinical stroke trials or been limited by side effects. Ongoing clinical trials with novel agents are trying to enroll a more homogeneous population of stroke patients in an effort to demonstrate treatment benefit.

Introduction

Neuroprotective agents are potential stroke treatments that try to preserve ischemic brain cells. Unlike thrombolytics such as tissue plasminogen activator (tPA) [1], they do not act on the thrombus that caused the stroke. The neuroprotective agents instead focus on the penumbra region of the stroke, where the cells are dysfunctional due to ischemia but not yet irreversibly injured like the cells in the core of the stroke. These therapies target events in the ischemic cascade to prevent ongoing injury to the ischemic brain.

When blood flow drops in the infarct core, the resultant depletion of adenosinetriphosphate (ATP) quickly causes failure of energy-dependent ion pumps and persistent cell depolarization. In the penumbra, recurrent depolarizations of the membrane potential may occur [2]. Neuroprotective agents that act on ion channels, such as BMS-204352 (Bristol-Meyers Squibb, Stamford, CT), may help to restore ion balance. If the recurrent depolarizations are allowed to persist, they can activate receptors that release calcium from the endoplasmic reticulum. By activating proteases, endonucleases, and phospholipases, elevated intracellular calcium levels cause damage to enzymes, DNA, and cell membranes. Many clinical trials have been performed with agents that have either modified the action of receptors, such as the N-methyl-D-aspartate (NMDA) receptor antagonists, or have blocked calcium channels in an effort to limit these injurious events. Moreover, the release of organic-bound iron catalyzes the formation of free radicals, providing a potential role for antioxidants and free radical scavengers in stroke treatment.

Unfortunately, return of blood flow to the ischemic region after lysis of the offending thrombus can actually contribute to additional injury, termed “reperfusion injury.” Leukocytes may directly occlude small vessels. When they reach the area of injury, the leukocytes release toxic products that lead to free radical and cytokine formation [3]. To promote these activities, leukocytes must first adhere to the vessel wall. This is mediated by receptors on both the neutrophil and the endothelium of the vessel. Monoclonal antibodies to leukocyte adhesion sites have been used to block this early step in reperfusion injury. Other neuroprotective agents stabilize membranes and promote neuronal healing.

Certain trials are investigating the use of lesion volume measurements, determined using magnetic resonance imaging (MRI), as surrogate markers for stroke outcome. Acute lesion volume is generally obtained using diffusion weighted imaging (DWI), an MRI technique that images slowly moving water protons such as those that have moved into the confined intracellular space when membrane pumps fail. Because it is able to show ischemia so early, DWI reveals ischemic changes before they are apparent on conventional MRI or computed tomography (CT) [4]. Because most DWI lesions expand, maximizing on the third day after symptom onset, lack of lesion expansion could be used as a measure of treatment efficacy [5]. In one analysis, if a lesion had 50% less expansion between days 1 and 3, the sample size required to show efficacy would be only 20 patients [5]. By allowing the selection of strokes in a certain location, neuroimaging offers the additional possibility of targeting a relatively homogeneous stroke population.

No neuroprotective agent has yet been found to be clinically efficacious despite significant research effort. In recent trials, an effort has been made to focus on the stroke

patients who are most likely to respond to the treatments. This article reviews the results of recent stroke treatment studies with neuroprotective agents and reports ongoing clinical trials with new agents.

Ion Channel Modulators

BMS-204352

Parallel clinical trials are currently in progress with the agent BMS-204352, which modulates ion flow across a transmembrane potassium channel, called a maxi-K channel. The agent theoretically promotes ion balance and allows restoration of cellular integrity. In an effort to target the patients in whom the potassium channels would be involved by the stroke, the clinical trials are limiting inclusion to patients with presumed cortical strokes of at least moderate severity. The studies are evaluating more than one dosage group within a 6-hour time window. An MRI substudy of acute lesion volume and brain perfusion is being conducted at selected sites. Early trials with BMS-204352 raised no safety concerns in either normal volunteers or stroke patients [6].

Calcium channel blockers

Because the excessive cellular calcium influx caused by ischemia can cause damage to enzymes, DNA, and cell membranes, drugs that block this effect have been heavily investigated for their potential as neuroprotective agents. These calcium channel blockers, however, have not shown efficacy in clinical acute stroke treatment. Patients in the most recently reported clinical trial, the Very Early Nimodipine Use in Stroke (VENUS) trial [7], received oral nimodipine within a therapeutic time window of 6 hours. After analysis of the first 439 patients predicted no beneficial effect of the drug, the trial was terminated [7].

Fosphenytoin

Fosphenytoin (Parke-Davis, Morris Plains, NJ) is a sodium channel blocker that prevents glutamate release. The same properties that have made the drug a useful anticonvulsant have been investigated for their ability to reduce neuronal damage in stroke. However, a multicenter trial in which patients received intravenous fosphenytoin or placebo within 4 hours of symptom onset showed no treatment benefit in an interim analysis. The trial was halted after the enrollment of 462 patients [8].

Lubeluzole

Lubeluzole (Janssen, Beerse, Belgium) blocks the sodium channel in cells and may have additional effects on nitrous oxide [9]. In two early studies of this drug, post hoc analyses suggested that treatment effects may be adversely affected by severe strokes and increased patient age. However, a subsequent large acute stroke trial with an 8-hour window was unable to show efficacy despite the exclusion of elderly patients with severe strokes. A meta-analysis of the three trials did not show treatment benefit [10]. A trial in which lubeluzole or placebo was given within an hour of tPA (which was stopped after the enrollment of 89 patients when no efficacy was demonstrated in the phase 3 trial) also failed to show any treatment benefit for lubeluzole [11]. Clinical stroke research with lubeluzole has now been abandoned.

Receptor Modulators

N-methyl-D-aspartate receptor antagonists

Early N-methyl-D-aspartate receptor antagonist trials

Although NMDA receptor antagonists were among the first neuroprotective agents to be investigated in clinical acute stroke trials, they were plagued by side effects that eventually terminated the trials. Like phencyclidine, which binds at a similar site, the NMDA receptor antagonists have caused hallucinations, agitation, and even hypotension [12]. Selfotel (Novartis, East Hanover, NJ) showed trends toward higher mortality in treated patients compared with the placebo cohort [13]. More recently, aptiganel Hcl (Boehringer Ingelheim, Rhein, Germany), assessed within 24 hours of stroke onset, displayed side effects of hypertension and central nervous system excitation [14]. One phase 2 trial with the NMDA antagonist C-101, 606, which is acquiring both imaging and clinical outcome data, is still in progress (Pfizer, New York, NY, unpublished data).

Glycine site N-methyl-D-aspartate receptor antagonists

To avoid the side effects associated with the NMDA antagonists, "indirect" NMDA receptor antagonists were developed. By hindering glycine from binding, these glycine-site NMDA receptor antagonists prevent glutamate from activating the receptor. Clinical trials have shown that this group of NMDA antagonists is well tolerated [15•,16,17]. However, efficacy for the agent GV150526 (Glaxo, Middlesex, UK) was not shown in a recent trial of 1804 patients performed in Canada, Europe, Australia, and Asia [17]. No difference was seen between the treatment and placebo groups in outcome at 3 months, measured by the Barthel, modified Rankin, or the National Institutes of Health Stroke Scale (NIHSS) scores. A second trial in the United States has also failed to find any treatment benefit [18].

Magnesium

Magnesium blocks the NMDA receptor and also appears to increase regional blood flow and antagonize voltage-sensitive calcium channels to reduce injury in ischemia [19]. In early studies, patients have tolerated the drug well [19–21]. An international phase 3 trial investigating the efficacy of magnesium in stroke, the Intravenous Magnesium Efficacy in Stroke (IMAGES) trial [22], is now in progress. Eligible patients must begin treatment (an infusion of 16 mmoL $MgSO_4$ over 15 minutes followed by 65 mmoL over 24 hours or placebo) within 12 hours from symptom

onset and also have limb weakness. An MRI substudy, MR IMAGES, is also planned.

Other receptor modulators

Nalmefene

Nalmefene (Baker Norton, Miami, FL) is an opioid antagonist with relative kappa-receptor selectivity. In a phase 3 clinical trial, the drug was administered within 6 hours of symptom onset to patients with an NIHSS score of 4 or more. A total of 368 patients were randomized. The primary endpoint required both a score of 60 or more on the Barthel Index and a moderate disability score or better on the Glasgow Outcome Scale at 12 weeks. Although nalmefene was well tolerated, the drug did not achieve significance for the primary endpoint [23•]. A secondary endpoint, looking for a treatment effect in patients under the age of 70 years, was also negative.

Clomethiazole

Clomethiazole (AstraZeneca, Wilmington, DE), a gamma-aminobutyric acid (GABA) agonist, increases the activity of inhibitory pathways. In Europe, the drug's inhibitory effects have been used to treat seizures and promote sedation. The first trial of clomethiazole in ischemia was performed in Europe, known as the Clomethiazole Acute Stroke Study (CLASS) study [24]. Patients received a 24-hour intravenous infusion of clomethiazole or placebo within 12 hours of symptom onset. As predicted by its central nervous system inhibitory properties, the drug's primary side effect was sedation. The trial was unable to show efficacy for improving functional outcome at 3 months.

A subgroup of patients, however, with higher cortical dysfunction, limb weakness, and visual field disturbances, called the Total Anterior Circulation Syndrome (TACS), in the European trial showed a 37% relative improvement. For this reason, a large phase 3 trial targeting patients with TACS strokes has now been completed in the United States. The drug was given within 12 hours of symptom onset (Astra Zeneca, unpublished data). Smaller arms of the study assessed drug safety in hemorrhagic strokes and in patients who also received tPA treatment. Results are expected in early 2001.

YM872 and Bay x3702

The agents Bay x3702 (Bayer, Pittsburgh, PA) and YM872 (Yamanouchi, Palo Alto, CA) are currently in earlier phases of study. Bay x3702, is a serotonin agonist acting at the 5HT1A receptor subtype. A multicenter trial of three dosage groups of Bay x3702 or placebo, given within 6 hours of symptom onset, has been completed [25]. YM872 is an alpha-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid (AMPA) antagonist. A dosage-finding trial with this agent has been completed without any safety concerns. A phase 3 trial was expected to begin in the fall, 2000.

Agents with Primary Effects During Reperfusion

Antiadhesion antibodies

Enlimomab

Enlimomab (Boehringer Ingelheim, Rhein, Germany)is a murine monoclonal antibody to the intercellular adhesion molecule (ICAM)-1 on the blood vessel endothelium that prevents the adherence of leukocytes to the vessel wall [26]. Enlimomab was assessed in a multicenter trial of over 600 patients, in which stroke patients received a first dose of intravenous enlimomab or placebo within 6 hours of symptom onset [27]. Unfortunately, the treated patients had worse outcomes and a higher mortality than those in the placebo group, perhaps related to the higher number of fevers in the treated group. Elevated temperatures have been previously found to worsen stroke outcome [28]. A possible explanation for the fevers was a potential immune response that the patients had developed to the murine antibody. No further trials with enlimomab are planned.

Hu23F2G

The agent Hu23F2G (ICOS, Bothell, WA) is a humanized antibody to CD11/CD18 leukocyte adhesion receptors. By blocking the leukocyte receptors, the drug Hu23F2G prevents the leukocyte from binding with ICAM-1 and other ligands. Because it is a humanized antibody, Hu23F2G should avoid potential immune responses that were of concern with enlimomab. A phase 2 trial did not show an increased incidence of serious adverse events or infections in Hu23F2G-treated ischemic stroke patients (n=32) compared with those in the placebo cohort (n=16) (ICOS, unpublished data). However, a phase 3 trial in patients with moderate to severe strokes has recently been suspended due to an "unfavorable safety to efficacy profile" by the safety committee (ICOS, unpublished data).

Membrane stabilization and healing

Citicoline

Citicoline (Interneuron, Lexington, MA) is an exogenous form of cytidine-5´-diphosphocholine (CDP-choline) that is used in membrane biosynthesis. It may help to minimize ischemic injury by stabilizing membranes and decreasing free radical formation. After a phase 2 trial showed improved outcome in stroke patients treated with either a dose of 500 mg or 2000 mg of citicoline [29], a phase 3 trial compared the 500-mg/d dose, given orally every day for 6 weeks, with placebo, randomizing patients in a 2:1 fashion [30]. Patients received the first dose within 24 hours of symptom onset. The study showed no overall benefit to treatment with citicoline (n=267) compared with placebo (n=127) on the prespecified assessments of functional outcome at 3 months. However, post hoc analyses suggested that citicoline might demonstrate benefit in a subgroup of patients with more severe strokes (NIHSS score greater than 8).

These post hoc analyses prompted the initiation of a large, phase 3 placebo-controlled trial of citicoline (2000 mg/d for 6 weeks). In an effort to target the subgroup predicted to best respond to the drug, this trial enrolled patients with moderate to severe deficits. The first dose was given within 24 hours of symptom onset. A 7-point improvement in NIHSS scores at 12 weeks was chosen as the primary outcome measure, based upon analyses of previous trials. The enrollment of 899 patients was completed in 1.5 years. Unfortunately, the trial did not meet its primary endpoint [31]. However, on a post hoc test, treated patients were more likely than the placebo cohort to have complete or nearly complete recovery on the Modified Rankin scale and showed a trend toward increased neurologic recovery on the NIHSS scale.

T2-weighted MR images, obtained in all patients at 3 months, did not show a significant difference in mean lesion size in the treated patients compared with the placebo cohort [32]. In an MRI substudy performed at selected sites, patients also received a baseline DWI exam. When those 90 patients' scans in whom the baseline cortical volume was between 1 and 120 cm^3 were assessed, the treatment group showed a lesser volume change from baseline and a smaller mean lesion volume at 3 months than the placebo group.

Basic fibroblast growth factor

Trafermin (Wyeth-Ayerst Research, Princeton, NJ), a basic fibroblast growth factor, has been postulated to regulate neuronal healing after ischemia. Although a phase 2 safety trial (Scios, Sunnyvale, CA) in which the drug was administered intravenously for up to 24 hours was only associated with a transient leukocytosis [33], a phase 3 trial (n=303) evaluating the efficacy of trafermin in stroke patients presenting within 6 hours of symptom onset found that trafermin treatment increased mortality and worsened outcome [34].

Free radical scavengers

Tirilazad mesylate (Pharmacia and Upjohn, Peapack, NJ), a free radical scavenger, could limit ischemic injury. However, phase 3 studies assessing a dose of 6 mg/kg of tirilazad per day for 3 days, starting within 6 hours of stroke onset, did not show efficacy [35]. When it was hypothesized that the lack of benefit could have been due to an inadequate dose, a subsequent study was done with a high-dose regimen (10 to 12.5 mg/kg/d in men, and 12 to 15 mg/kg/d in women). Patients were treated within 4 hours of symptom onset. After 126 patients were enrolled, the study was prematurely stopped because questions of safety emerged from a parallel study in Europe. Except for drug-related infusion site disorders, the high doses of tirilazad were reasonably well tolerated in the American study [35].

Antioxidants

Antioxidants, which may help to scavenge free radicals as well as reduce inflammation, are gaining interest as clinical stroke treatment agents. A phase 2 trial with a novel antioxidant, NXY059, is projected to begin in early 2001 (Astra Zeneca, unpublished data), and investigations of other, similar agents are planned.

Table 1. List of neuroprotective agents in (or soon to begin) clinical trials

Ion channel and receptor modulators
- Maxi-K channel modulator (BMS-204352)
- GABA antagonist (clomethiazole)
- AMPA antagonist (YM872)
- NMDA antagonist (C-101,606)
- NMDA antagonist (magnesium)
- Serotonin agonist (Bay x3702)

Agents acting during reperfusion
- Antioxidant (NXY059)
- Antioxidant and antiplatelet agent (ginkgo biloba)

AMPA–alpha-amino-3-hydroxy-5-methylisoxazole-4 proprionic acid; GABA–gamma-aminobutyric acid; NMDA–N-methyl-D-aspartate.

Conclusions

Although many multicenter, phase 3 clinical trials investigating potential neuroprotective agents for stroke have shown either a lack of efficacy or unacceptable side effects, innovative research in this field continues (Table 1). Despite the discontinuation of most studies of NMDA receptor antagonists because of side effects, trials investigating drugs that modulate other receptors, such as the GABA, AMPA, or serotonin receptors, are still in progress. Calcium channel blockers have not shown efficacy, but an agent that opens potassium ion channels is in phase 3 trials. Of those neuroprotective agents that exhibit their primary effects during the reperfusion phase of ischemia, the leukocyte adhesion blockers have shown negative results, but antioxidant therapy holds promise. Large trials will begin to evaluate antioxidant treatment in clinical stroke shortly.

The lack of clinical efficacy shown by the neuroprotective agents has been disappointing relative to the promise of earlier animal studies. The 6-hour treatment window used in human studies compared with the 1- or 2-hour window in animal studies provides a potential reason for this difference. The variability of human strokes represents one of the largest obstacles to showing treatment benefit. Mild strokes can mask treatment effects by producing excellent outcomes in both the treatment and placebo groups, whereas severe strokes can cause disproportionately large numbers of poor outcomes. Receptor-rich cortical tissue, upon which some neuroprotective agents act, is not involved by ischemia in all stroke patients. Current studies have restricted inclusion to patients with cortical signs or other specified findings, or to those with NIHSS scores within a certain range. The variability of clinical stroke makes it likely that treatment effects will be small. Studies

need to be powered accordingly. The use of newer MRI techniques may be able to assist in the selection of a homogeneous patient population and in measuring efficacy.

Effects of a single agent on the ischemic cascade may not be sufficient to stem the tide of cellular destruction. Consideration will need to be given to large studies of thrombolytic and neuroprotective drugs used in combination, and even to combinations of neuroprotective agents [36]. Finally, the drugs will need to be given within a short therapeutic time window to enhance the potential for efficacy. By targeting suitable clinical candidates and continuing drug development, the study of neuroprotective agents in stroke treatment will likely produce an efficacious agent.

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