

An Overview of Acute Stroke Therapy

Past, Present, and Future

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The effort to develop effective therapies for acute ischemic stroke achieved several important successes during the past decade, but also many disappointing failures. The 2 primary successes were related to thrombolysis. The first was the NINDS rt-PA (National Institute of Neurological Disorders and Stroke Recombinant Tissue-Type Plasminogen Activator) trial reported in 1995. This study demonstrated that initiation of intravenous (IV) rt-PA within 3 hours after the onset of acute ischemic stroke significantly improved outcome at 3 months.¹ This study led to the approval of rt-PA initiated within 3 hours of stroke onset as the only currently available acute stroke therapy. The second major success was the demonstration that intra-arterial prourokinase initiated within 6 hours of stroke onset in patients with angiographically documented proximal middle cerebral artery (MCA) occlusion also improved outcome at 3 months.² A third marginally positive acute stroke trial used ancrod, a defibrinogenating agent derived from Malaysian pit vipers.³ Ancrod initiated within 3 hours after stroke onset also improved 3-month outcome but to a lesser degree than either rt-PA initiated within 3 hours or prourokinase initiated within 6 hours. These successful acute stroke therapy trials were outweighed by a large number of neuroprotective trial failures. Currently, not one of many purported neuroprotective therapies assessed in pivotal clinical trials has demonstrated unequivocal, statistically significant improvement in clinical outcome.⁴ The neuroprotective trials all included patients who presented with a stroke 3 hours after onset, and the therapies used for each patient failed for myriad reasons that will be explored in detail.

In this overview of the current status and future direction of acute stroke therapy, we will discuss in detail the current situation of thrombolytic therapy for acute ischemic stroke, reviewing the results of published clinical trials, postmarketing experience with rt-PA given within the 3-hour window, and future directions of how to potentially expand this window for IV thrombolytic therapy. The status of various neuroprotective therapies for acute ischemic stroke will be reviewed and potential new neuroprotective strategies previewed. Last, we attempt to envision likely approaches toward multiple therapeutic in-

terventions, a treatment strategy likely to lead to maximal improvement in the greatest number of stroke patients.

THROMBOLYTIC THERAPY FOR ACUTE ISCHEMIC STROKE

The NINDS rt-PA trial was the first acute ischemic stroke trial to unequivocally demonstrate that this disorder could be benefited by any therapeutic intervention.¹ In this trial, 624 carefully selected patients were randomly and blindly assigned to therapy with rt-PA (0.9 mg/kg) or placebo within 3 hours of stroke onset. Half of the patients were treated within 90 minutes of onset, an accomplishment by the investigators participating in the trial. The patients treated with rt-PA had an abso-

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Postmarketing Studies of Intravenous rt-PA Studies Compared With the NINDS Trial*

Study	Patients, No.	NINDS Trial					
		Median NIHSS	Age, y, Mean ± SD	Median Time to Treat, min	Modified Rankin Score 0-1 at 90 Days, %	Mortality, %	Symptomatic ICH, %
NINDS, placebo ¹	165	15	66 ± 13	90	26	21	1
NINDS, rt-PA (part 2) ¹	168	14	69 ± 12	90	39	17	7
Chiu et al ⁷	30	14 ± 8	66 ± 15	157	30	23	7
Egan et al ⁸	33	17	NA	157	36	18	9
Tanne et al ⁹	75	NA	66 ± 15	NA	34 (At discharge)	11	3
Wang et al ¹⁰	14	15.4 ± 2	(15-88)	NA	57	7	7
Grond et al ¹¹	100	12	63 ± 11	124	40	12	5
Albers et al ¹²	296	13	68 ± 13	165	35 (Day 30)	12	4

*rt-PA indicates recombinant tissue-type plasminogen activator; NINDS, National Institute of Neurological Disease and Stroke; NIHSS, National Institutes of Health Stroke Scale Score; ICH, intracerebral hemorrhage; and NA, not available.

lute improvement rate of 11% to 13% at 90 days when compared with the placebo patients on various outcome measures that evaluated both neurologic and functional status. The patients treated with rt-PA had a symptomatic intracerebral hemorrhage rate of 6.4% (almost half the patients died) within 36 hours of onset, while the rate was only 0.6% in the placebo group. Despite this early hemorrhagic risk, the 90-day mortality rate was 17% in the rt-PA group and 21% in the placebo group. Subsequent analysis of the study data demonstrated that early computed tomographic (CT) demonstration of extensive edema or hypodensity, history of diabetes mellitus, and elevated baseline National Institutes of Health Stroke Scale Score (NIHSS) were predictors of poor outcome.⁵ The use of rt-PA was associated with improved outcome in all stroke subtypes included in the study, in patients across the broad range of baseline stroke severity, and in all age groups. The initial analysis of the study data did not distinguish a difference in benefit of rt-PA related to time-of-treatment initiation. However, in a subsequent analysis that adjusted for baseline severity of the neurologic impairment, an earlier time to initiation of therapy was associated with a more favorable outcome, demonstrating an inverse linear relationship between time to treat and the odds ratio of a favorable outcome.⁶ The confidence interval for a favorable outcome crossed 1 in patients treated beyond 2 hours 40 minutes after stroke onset, suggesting that treatment initiated beyond

this time point may not be of proven efficacy.

Several postmarketing studies of IV rt-PA are now available.⁷⁻¹⁵ Patients were included in these studies using the general guidelines for treatment used in the NINDS trial. The most important inclusion criterion was initiation of therapy within 3 hours of stroke onset. Most of the studies encompassed relatively small numbers of patients, ranging from 14 to 75 (**Table**). However, several larger studies are available, including the study reported by Grond and colleagues¹¹ of 100 patients and the STARS (Standard Treatment With Activase to Reverse Stroke) study of 296 patients.¹² The median time from stroke onset to initiation of rt-PA therapy ranged from 124 minutes in the study by Grond and colleagues to 165 minutes in the STARS study. The percentage of patients achieving a modified Rankin score of 0-1, the results defined as a favorable outcome in the NINDS trial, ranged from 34% to 57%; although, in several of the reports, day 90 data were not provided.

On the surface, the rates of favorable functional outcome demonstrated in these postmarketing studies appear to be quite good, surpassing in some studies the 39% 0-1 Rankin rate at 90 days seen in the NINDS trial. These results must be interpreted cautiously because the baseline severity of the patients treated in these postmarketing studies were not as severe as in the NINDS trial. For example, in the 2 largest postmarketing studies, the study by Grond and colleagues and the STARS

study, the median baseline NIHSSs were 12 and 13, while in the NINDS trial the median baseline NIHSS in the placebo group was 14 in part 1 and 15 in part 2. In other acute stroke trials where the baseline NIHSS was 11, the percentage of patients achieving a Rankin score of 0-1 approximates 37%¹³ and when the baseline NIHSS was 13, 29% achieved this outcome.¹⁴ Comparing the outcomes in the study by Grond and colleagues and the STARS study with those of a placebo group that had a similar degree of baseline severity demonstrates an absolute improvement rate of 3% to 6%, not the approximately 12% absolute rate of improvement observed with rt-PA treatment in the NINDS trial. The postmarketing studies do, however, provide some encouraging data about the rate of symptomatic intracerebral hemorrhage. The percentage of patients experiencing this serious complication of thrombolysis ranged from 0% to 19% with only 2 studies observing double-digit rates of intracerebral hemorrhage. In the 2 largest studies, the intracerebral hemorrhage rates were only 4% to 5%. It therefore appears that expanding IV rt-PA use into general practice is not associated with a substantially increased risk of intracerebral hemorrhage, if the guidelines for patient selection used in the NINDS trial are followed.

Studies evaluating the efficacy of IV rt-PA beyond the 3-hour time window were conducted. The first clinical trial to evaluate IV rt-PA up to 6 hours after stroke onset was the European Cooperative Acute Stroke

Study (ECASS-1).¹⁴ Patients were randomly and blindly assigned to rt-PA (1.1 mg/kg) or placebo within the 6-hour period after acute stroke onset in the MCA territory. Predefined exclusion criteria included evidence of CT hypodensity or sulcal effacement involving more than a third of the MCA territory on the pretreatment CT scan. The overall results as analyzed in the intention-to-treat analysis of the trial were negative, but when protocol violators were excluded, several outcome measures were better in the rt-PA group. The CT exclusion criterion (more than a third of the MCA territory showing early infarct signs) occurred in 63 (58%) of 109 protocol violators and these patients had a high risk of symptomatic often-fatal intracerebral hemorrhages when they received rt-PA. A second ECASS study was performed using the NINDS dose of rt-PA, 0.9 mg/kg, and, in this study, the study investigators received better CT training to identify hyperacute CT changes indicative of early infarction.¹³ Patients were again randomized up to 6 hours after stroke onset. In ECASS-2, the primary end point was the percentage of patients in the 2 treatment groups achieving a Rankin score of 0-1, and this outcome was observed in 40.3% of the rt-PA-treated group and 36.6% of the placebo group, a nonsignificant difference. The median baseline NIHSSs were only 11 in the 2 groups. Therefore, the baseline severity of the stroke patients included in ECASS-2 was less than in the first ECASS study or the NINDS study, likely explaining in part the better outcome observed in the placebo group of this trial than in the other 2 IV thrombolysis trials. A post hoc analysis of the ECASS-2 data demonstrated a significant difference between the rt-PA-treated group and the placebo group when the Rankin score was dichotomized into 0 to 2 and greater than 2. In this analysis, 54.3% of the rt-PA patients achieved a 90-day outcome of 0 to 2, while only 46% of placebo patients had a 90-day Rankin score of 0 to 2. The difference between a score of 1 or 2 on the Rankin scale is not great and this result speaks to the inherent difficulties in determining the best outcome measure to use in acute stroke studies.

One other large IV rt-PA study, the Alteplase Thrombolysis for Acute Non-Interventional Therapy in Ischemic Stroke (ATLANTIS), evaluating therapy initiated from 3 to 5 hours after stroke onset is available. In this study, the patients received 0.9 mg/kg of IV rt-PA and the primary outcome measure was the percentage of patients achieving a NIHSS of 0 or 1 at 90 days.¹⁵ The study included 547 patients and the primary end point was almost identical in the 2 groups. The median baseline NIHSS was 11 in the 2 groups and a day 90 modified Rankin of 0-1 was achieved in 42% of the rt-PA group and 40% of the placebo patients. One positive result from the ECASS-2 and ATLANTIS trials was that the rate of symptomatic intracerebral hemorrhage was 8.8% and 7.0%, respectively, not greatly increased from the 6.4% rate seen with rt-PA in the 3-hour window NINDS trial.

The Intra-arterial PROACT-2 (Recombinant Prourokinase in Acute Cerebral Thromboembolism) study was a 0- to 6-hour thrombolysis trial that demonstrated a significant treatment effect. This study used recombinant prourokinase (r-proUK) delivered locally into an angiographically documented proximal MCA thrombus with low-dose IV heparin.² In both the active treatment and placebo groups, the median time to treat in PROACT-2 was 5.3 hours and the median baseline NIHSS was 17. The trial included 180 patients randomized 2:1 to r-proUK or placebo. At day 90, 40% of the patients treated with r-proUK achieved the primary outcome measure of a Rankin score of 0 to 2, while only 25% of the placebo patients achieved this favorable outcome ($P = .04$). Secondary outcome measures also tended to be better in the r-proUK group. Symptomatic intracerebral hemorrhage within 24 hours occurred in 10.2% of the r-proUK group and 1.9% of control patients. Despite the early risk of symptomatic intracerebral hemorrhage, the 90-day mortality was almost identical in the 2 groups, 25% in the r-proUK group and 27% in controls. The PROACT-2 study demonstrates that thrombolytic therapy can be effective when initiated up to 6 hours after stroke onset in care-

fully selected patients and should initiate additional attempts to successfully expand the time window for IV thrombolysis in acute ischemic stroke.

Currently, with the restrictive 3-hour time window necessary for the use of IV rt-PA in stroke therapy only approximately 1% to 2% of acute stroke patients are estimated to receive this intervention.¹⁶ While patient educational efforts and institutional initiatives might increase the percentage of patients treated to perhaps 5% to 10%, expanding the time window for successful IV thrombolysis should have a much greater impact on the number of patients treated. How might the therapeutic time window for beneficial IV thrombolysis be expanded in acute ischemic stroke? There are at least 2 strategies possible that may be synergistic. The first strategy is better identification of patients likely to respond to treatment beyond 3 hours after stroke onset. In PROACT-2, the use of angiography helped to identify patients and likely led in part to the success of the trial.² Unfortunately, angiography is time-consuming and not readily available at many institutions throughout the day. In addition, angiography only provides information about the presence or absence of a vascular occlusion and does not provide information about the status of ischemic injury within the brain parenchyma.

The new magnetic resonance imaging (MRI) techniques of diffusion and perfusion MRI combined with magnetic resonance angiography can provide a wealth of information about the extent and location of ischemic injury and the status of perfusion in the microvasculature, and document the presence or absence of an occlusion in the major intracerebral vessels.¹⁷ These MRI techniques can be performed in the same approximate time required to obtain a CT scan and are becoming widely available. Preliminary evidence suggests that acute stroke patients who have perfusion lesion volumes larger than diffusion lesion volumes, so-called diffusion-perfusion mismatch have ischemic regions within the mismatch that are more likely to respond to therapeutic interventions such as thromboly-

sis.¹⁸ Several studies demonstrated that approximately 70% of acute stroke patients evaluated within 6 hours of stroke onset demonstrate this pattern of perfusion-diffusion mismatch.^{19,20} Follow-up studies in patients with a diffusion-perfusion mismatch document that the diffusion volume expands into the region of the perfusion abnormality in most patients who are left untreated. In 1 preliminary study, patients with a diffusion-perfusion mismatch who were successfully recanalized with IV rt-PA had a much better clinical outcome than patients who did not reperfuse.¹⁸ Further studies are needed to document if patients with a diffusion-perfusion mismatch observed 3 to 6 hours after stroke onset are good candidates for IV rt-PA.

Concerns arose that MRI may not accurately detect acute intracerebral hemorrhage. However, several recent reports document that susceptibility-weighted MRI studies can reliably demonstrate hemorrhages.^{21,22} It will therefore be likely that CT scans will not be required when screening patients for inclusion in MRI-based clinical trials with IV thrombolysis.

A second potential approach to prolonging the therapeutic time window for successful thrombolysis would be to give neuroprotective therapy before, during, or after the infusion of IV rt-PA. Neuroprotective therapy initiated before or during the use of IV rt-PA could extend the time that the ischemic penumbra, the presumed therapeutic target for both thrombolysis and neuroprotection, remains salvageable.²³ Animal studies confirm that neuroprotective therapy can prolong the time window for successful reperfusion.²⁴ Currently, there is no evidence that rt-PA and a neuroprotective drug used in combination act synergistically to extend the time window for IV thrombolysis in stroke patients, but such combination trials are being considered. Another potential therapeutic combination would be the use of IV rt-PA followed by an agent designed to inhibit reperfusion injury induced by successful clot lysis. The possibility of secondarily generated reperfusion injury in the brain after clot

lysis has been raised in the past without direct confirmatory evidence.

Recently, both animal and human MRI studies demonstrated that secondary injury after successful reperfusion does indeed occur, although the precise mechanisms responsible remain to be elucidated.^{25,26} The most likely processes responsible for secondary injury after successful reperfusion are the recruitment and activation of inflammatory white blood cells (primarily polymorphonuclear leukocytes), the release of cytokines, the generation of oxygen free radicals and apoptosis. It is quite possible that more than one of these potential inducers of secondary injury are active within different regions of the reperfused tissue. Prior animal studies demonstrated that drugs inhibiting polymorphonuclear leukocyte activity or free radical scavengers are almost exclusively beneficial in reperfusion models and demonstrate little if any effect in permanent occlusion models.⁴ Combining these types of drugs with thrombolysis in animals does indeed extend the therapeutic time window for successful reperfusion.²⁷ Using these types of drugs after documented reperfusion induced by IV rt-PA could be another way to extend the time for beneficial thrombolysis in stroke patients, presumably by inhibiting reperfusion injury that would impact on outcome measures determined several months after stroke onset.

PATHOPHYSIOLOGICAL TARGETS FOR NEUROPROTECTION

Neuroprotective agents and strategies have been studied for years and appear to be effective in a variety of preclinical stroke models. However, none of the drugs have proven conclusively to be effective in humans. It is difficult to translate data regarding drug dosage, time window, sex differences, and, in particular, the stroke target population from animals to humans. Misunderstanding and misinterpretation of these issues may have caused negative trial results and a pessimistic view for neuroprotection of stroke in general. Evidence from the recent clinical trials demonstrates that subpopulations of

stroke patients may benefit from the neuroprotective approach. It is hoped that the first neuroprotectant will be proven to be effective in the near future.

Compared with thrombolysis, the neuroprotective approach for stroke treatment is more complex and reflects the diversity of the ischemic cascade (**Figure 1**). Neuroprotective agents have been developed and tested for nearly all components of the ischemic cascade. As seen with recently discovered mechanisms such as gene expression or the role of zinc after stroke, new agents may be developed and new therapeutic options occur. The discovery that calcium-induced excitotoxicity occurs after ischemia is relatively old (about 20 years) and was widely accepted as a key event after cerebral ischemia. Ischemia-induced energy failure causes membrane depolarization and release of excitatory amino acids such as glutamate into the extracellular space.^{28,29} Glutamate receptors become activated, resulting in calcium overload of neuronal cells. This step can effectively be blocked by *N*-methyl-D-aspartate (NMDA), and α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) channel antagonists. Water, sodium, and chloride move intracellularly via monovalent ion channels into the cell causing so-called cytotoxic edema. The large amount of intracellular calcium activates proteolytic enzymes that degrade cytoskeletal and extracellular matrix proteins. Calcium also activates phospholipase A2 and cyclooxygenase producing oxygen free radicals. Nitric oxide (NO) is synthesized from L-arginine and molecular oxygen by calcium-dependent NO synthase (NOS) and NO reacts with superoxide to form peroxynitrite. The free radical release promotes further membrane damage, and subsequently mitochondrial dysfunction. Free radical activity can be blocked by antioxidants and free radical scavengers.

Secondary to these events, expression of proinflammatory genes is induced by the synthesis of transcription factors and release of mediators of inflammation, such as platelet-activating factor, tumor necrosis factor, and interleukin 1 β (IL-1 β).³⁰ Consequently, expression of the ad-

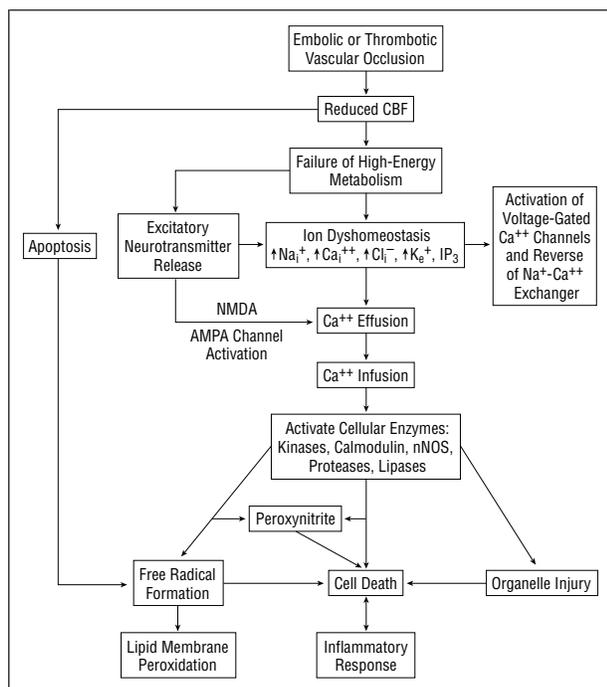


Figure 1. The cascade of events currently thought to be important contributors to focal ischemic brain injury. CBF indicates cerebral blood flow; Na^+ , intracellular sodium; Ca^{++} , intracellular calcium; Cl^- , intracellular chloride; K^+ , extracellular potassium; IP_3 , inositol 1,4,5-triphosphate; NMDA, N-methyl-D-aspartate; AMPA, α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; and nNOS, neuronal nitric oxide synthase.

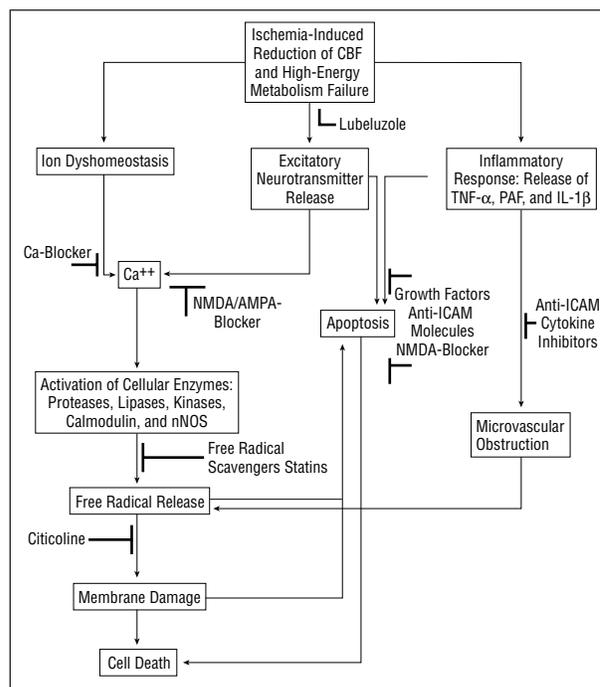


Figure 2. Diagram depicting where various neuroprotective agents may intervene on the ischemic cascade. $\text{TNF-}\alpha$ indicates tumor necrosis factor α ; PAF, platelet-activating factor; $\text{IL-1}\beta$, interleukin 1β ; and ICAM, intercellular adhesion molecule. Other abbreviations are given in the legend to Figure 1.

hesion molecules intercellular adhesion molecule-1 (ICAM-1), P-selectins and E-selectins occur on the endothelial surface.³¹⁻³³ After binding to adhesion molecules, neutrophils adhere to the endothelium, cause microvascular obstruction, cross the vascular wall, and enter the brain parenchyma followed by macrophages and monocytes. Blocking of adhesion molecules can prevent these events. Activated inflammatory cells and injured neurons produce a number of toxic mediators that may worsen ischemia. For example, infiltrating neutrophils produce toxic amounts of NO through activation of inducible NOS (iNOS).³⁴ Pharmacological blockade of iNOS inhibitors consequently reduces ischemic brain injury.³⁵ Ischemic neurons also express cyclooxygenase 2, an enzyme that mediates ischemic injury by producing superoxide and toxic prostanoids.³⁶ Inhibition of the enzyme by cyclooxygenase 2-blockers significantly reduces ischemic brain damage. Furthermore, injured neurons produce the proinflammatory cytokine, $\text{IL-1}\beta$, that can be blocked by $\text{IL-1}\beta$ inhibitors to reduce infarct size after ischemia.³⁷

Another mechanism that contributes to ischemic injury is apoptosis, although the role of apoptotic injury in stroke patients remains uncertain. Triggered by a number of pathophysiological processes, including excitotoxicity, free radicals, the inflammatory reaction, and mitochondrial and DNA damage, apoptosis occurs after milder ischemic injury, particularly within the ischemic penumbra.³⁸ Apoptosis is mediated by a cascade of gene expression, including the caspases, a family of aspartate-specific cysteine proteases, as well as genes that suppress (eg, Bcl-2) or augment (eg, Bax) cell death.^{39,40} Caspase activity can be blocked by caspase inhibitors, reducing infarct size and apoptosis as well as improving outcome.^{41,42} Other agents that may counteract apoptosis after focal cerebral ischemia via Bcl-2/Bax-dependent mechanisms are growth factors such as brain-derived neurotrophic growth factor.⁴³

Recovery and reorganization of the brain after focal ischemic injury occurs over weeks and may have a major impact on the outcome after stroke.^{44,45} The underlying mechanisms of recovery in-

clude neuronal sprouting and synaptogenesis, which are part of the spontaneous recovery process particularly after smaller focal lesions. Enhancement of recovery followed by improvement of behavioral outcome can be achieved in animals with drugs such as growth factors and amphetamines.^{46,47}

ACUTE TREATMENT STRATEGIES

Various neuroprotective agents that may intervene on the ischemic cascade are presented in **Figure 2**.

Calcium Antagonists

Calcium-channel antagonists were among the first drugs evaluated for neuroprotection after stroke. They reduce calcium influx into the cell via voltage-sensitive calcium channels. Calcium antagonists were indeed shown in several experimental studies to be neuroprotective after focal cerebral ischemia.⁴⁸ The best studied drug is the dihydropyridine compound nimodipine, which typically blocks the L-type calcium channel. Nimodipine was tested in at least 10

randomized, placebo-controlled stroke trials. Aside from the positive results with reduction of mortality and improved neurologic outcome at 6 months after stroke in an early trial, all other trials had negative results.⁴⁹ Adverse effects of IV nimodipine include hypotension, which was directly correlated to an increase in mortality.⁵⁰ A meta-analysis of 9 trials including 3719 patients showed a benefit for oral nimodipine (30 mg over 6 hours) in the subgroup treated within 12 hours of stroke onset.⁵¹ Therefore treatment with oral nimodipine was again studied within 6 hours of symptom onset in the Very Early Nimodipine Use in Stroke (VENUS) trial.⁵² In the VENUS study no benefit on outcome after stroke was observed. Nevertheless, nimodipine is standard treatment for prevention of ischemic neurologic deficits after subarachnoid hemorrhage.⁵³

NMDA-Antagonists

The NMDA antagonists reduce calcium influx into neurons through postsynaptic agonist-operated calcium channels. They were the first drugs that remarkably reduced infarct size (40%-70%) after experimental focal cerebral ischemia, primarily in the ischemic penumbra.^{54,55} This effect can be achieved with competitive and noncompetitive NMDA antagonists. Competitive NMDA antagonists such as phosphonates or selfotel block the glutamate recognition site of the receptor. Noncompetitive NMDA antagonists, including phencyclidine, ketamine, dizocilpine maleate, dextrorphan hydrochloride, and cerestat, block the NMDA-associated ion channel in a use-dependent manner. Negative modulation of receptor activity can be achieved by zinc and hydrogen. Magnesium blocks the channel in a voltage-dependent manner and has been shown to reduce infarct size after focal cerebral ischemia. Apart from the main recognition site for glutamate, the receptor also contains a glycine site and inhibition of the glycine action reduces NMDA receptor activity. Antagonists of the glycine site of the receptor also reduce infarct size after experimental focal cerebral ischemia.^{56,57} Although pre-

clinical studies in a variety of species demonstrated potent infarct reducing effects, clinical trials using NMDA antagonists (selfotel, eliprodil, cerestat, dextrorphan) were negative or stopped due to adverse effects.⁵⁸⁻⁶¹ Adverse effects with some of these drugs occurred before a neuroprotective plasma level could be achieved. Adverse effects occurred in a dose-dependent manner and included neuropsychiatric symptoms (agitation, confusion, hallucination, catatonia, ataxia, dysarthria) and hypertension. These adverse effects are known to be phencyclidine related and should not occur, when targeting another subunit of the NMDA receptor. Indeed, antagonists of the glycine site of the NMDA receptor (GV150526, ACEA1021) are generally better tolerated without neuropsychiatric adverse effects.^{62,63} A phase 3 trial of GV150526 was completed and the results were negative.⁶⁴ Another compound that acts on the NMDA receptor and currently is being studied in a multicenter clinical trial is magnesium. A pilot study showed that the drug was well tolerated and associated with a trend toward fewer early deaths in a magnesium-treated group.⁶⁵

AMPA-Antagonists

AMPA-receptor antagonists prevent sodium influx into the cell by blockage of the AMPA/kainate receptor and prevent cell depolarization and the subsequent intracellular calcium overload of the cell. The AMPA receptor antagonists such as NBQX and ZK200775 have potent neuroprotective capacities when given after experimental focal cerebral ischemia⁶⁶ but adverse effects include nephrotoxicity (NBQX) and sedation (ZK200775).

γ -Aminobutyric Agonists

Enhancing the activity of the γ -aminobutyric acid A subtype receptor is another mechanism for inducing neuroprotection.⁶⁷ This inhibitory neurotransmission system hyperpolarizes and stabilizes resting membrane potential and may therefore inhibit peri-infarct-depolarizing events, a phenomenon that is associated with infarct evolution in ani-

mal stroke models.⁶⁸ The γ -aminobutyric acid agonists clomethiazole and muscimol reduce infarct size in animals.^{69,70} Clomethiazole was studied in a European phase 3 trial and the overall results were negative.⁷¹ However, patients with total anterior circulation infarcts who represented 40% of the study population showed an 11% absolute improvement rate on the primary functional outcome measure, the Barthel Index. The main adverse events associated with clomethiazole use were somnolence and rhinitis. Based on these encouraging results, a second efficacy trial restricted to patients with large anterior circulation strokes was organized and will be completed soon.

Lubeluzole

Lubeluzole, a benzothiazole compound, is a sodium blocker that prevents the presynaptic glutamate release and reduces postsynaptic excitotoxicity. Lubeluzole also prevents glutamate-mediated increases in NO production by inhibiting NOS activity. Experimental studies demonstrated neuroprotective effects after focal cerebral ischemia.^{72,73} Two large clinical, multicenter, and placebo-controlled trials, 1 in Northern America and 1 in Europe-Australia, have been completed.^{74,75} Besides occasional and transient electrocardiographic QT prolongations, lubeluzole was well tolerated at the concentrations given in both trials (7.5 mg/h IV followed by 10 mg/d for 5 days IV). However, there was no significant effect on the primary end point mortality, although the American trial^{74,75} showed a significant 7% increase in patients who had little or no disability at 3 months after stroke. A meta-analysis of 1375 patients suggested a positive effect on mild-moderate strokes, but no effect on severe strokes.⁷⁶ Therefore, a third trial (LUB-INT-13) was performed, designed to detect an approximately 7% functional benefit in the active treatment group.⁷⁷ The results were negative and development was stopped.

Free Radical Scavengers

Tirilazad mesylate, a 21-aminosteroid, acts as a free radical scavenger and has

antioxidant effects. Tirilazad treatment reduces infarct size after transient but not after permanent focal cerebral ischemia. Tirilazad was tested in several clinical trials with inconclusive results. The drug was well tolerated at a daily dose of 6 mg/kg.^{78,79} The RANTTASS (Randomized Trial of Tirilazad Mesylate in Patients With Acute Stroke) trial was terminated for lack of efficacy.⁸⁰ Release of NO and peroxynitrite can be inhibited by neuronal NOS or inducible iNOS blockers. The selective neuronal NOS blocker 7-nitroindazole and 1-(2-trifluoromethylphenyl) imidazole significantly reduced infarct size after focal and global cerebral ischemia in animals.^{81,82} Blockage of iNOS can be selectively achieved by aminoguanidines. Aminoguanidines were reported to be potent neuroprotectants after focal cerebral ischemia.⁸³ Aminoguanidines seem even to be protective when the treatment is delayed for 24 hours, making them interesting for future human use.⁸⁴

Another promising antioxidant is the seleno-organic compound ebselen. Ebselen acts through a glutathione peroxidase-like effect. It inhibits the peroxidation of membrane phospholipids and lipoxygenase in the arachidonate cascade. Ebselen also blocks the production of superoxide anions by activated leukocytes, inhibits iNOS, and protects against peroxynitrite. Ebselen has been shown to be neuroprotective after transient and permanent experimental focal cerebral ischemia.^{85,86} A clinical pilot study showed that oral ebselen (300 mg/d) was well tolerated and significantly improves functional outcome when given within 24 hours of stroke.⁸⁷ Given within 12 hours (150 mg/d) after ischemia, there was a trend toward reduced infarct volume and better outcome in patients treated with ebselen. A significant reduction in infarct volume and outcome occurred only in the 6-hour subgroup.⁸⁸

Antibodies to Intercellular Adhesion Molecules

Monoclonal antibodies against the ICAM-1 receptor on the vascular endothelium prevent leukocyte activation and plugging. The ICAM-1

antibodies were shown to reduce infarct size and improve outcome after transient but not permanent experimental focal cerebral ischemia.⁸⁹ The results of a randomized, placebo-controlled trial (625 patients) using the anti-ICAM antibody enlimomab were negative. The treatment group received IV 160 mg the first day, 40 mg the next 4 days (time window 6 hours), and had an even worse outcome and increased mortality due to higher rates of fever, infection, and pneumonia.⁹⁰ The adverse effects were thought to be due to a complement-mediated reaction triggered by enlimomab.

Inhibition of Cytokines

The best-studied cytokines that have key roles after ischemia and that can be pharmacologically inhibited are IL-1 β and tumor necrosis factor α . IL-1 β mediates excitotoxicity through NMDA receptor activation and activates surface adhesion molecules.⁹¹ Consequently, overexpression of endogenous IL-1 β receptor antagonists or treatment with the IL-1 β antagonist zinc protoporphyrin reduces infarct and edema size with temporary focal ischemia.^{92,93} Another cytokine, TFN- α may also exacerbate ischemic and in particular reperfusion injury.⁹⁴ Inhibiting tumor necrosis factor α improves cerebral blood flow and reduces infarct size after focal cerebral ischemia.⁹⁵ Other cytokines involved in the reperfusion damage after ischemia are the IL-1 and IL-6, platelet activating factor, and transforming growth factor- β . As indicated by preclinical studies, inhibition of these cytokines may have neuroprotective effects after cerebral ischemia.⁹⁶⁻⁹⁸ Further experimental studies evaluating dosage and timing of cytokine antagonists in different models of focal ischemia and in different species need to be completed before future clinical development.

Statins

Recent trials of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) demonstrated a significant reduction of ischemic stroke incidence in patients with history of coronary ar-

tery disease.^{99,100} Since this reduction was independent of the serum cholesterol lowering, statins may have other effects besides their antiatherosclerotic properties. Prophylactic statin therapy in animals improves cerebral blood flow through up-regulation of endothelial NOS and reduces infarct size (30%).¹⁰¹ Statins also inhibit the cytokine-mediated (IL-1 β , tumor necrosis factor α) up-regulation of iNOS and production of NO in rat astrocytes and macrophages.¹⁰² Furthermore, statins may reduce lipoprotein oxidation and attenuate free radical injury.^{103,104} More studies are needed to define time window, dosage, and mechanisms of action before statins should be considered for a future clinical use in stroke.

RECOVERY TREATMENT

Growth Factors

Growth factors are endogenously occurring polypeptides that have not only neuroprotective but also regenerative and proliferative capacities and may therefore be unique candidates for stroke therapy. Several growth factors were shown to be neuroprotective after experimental ischemia in vivo and in vitro. The best-studied growth factors after focal cerebral ischemia are basic fibroblast growth factor,¹⁰⁵ brain-derived neurotrophic growth factor,¹⁰⁶ insulin-like growth factor,¹⁰⁷ and osteogenic protein-1.¹⁰⁸ They are all robust neuroprotectants after acute stroke and reduce infarct size 35% to 50% in animals. Potential mechanisms of action after stroke include attenuation of excitotoxicity, improvement of cerebral blood flow, and reduction of apoptosis.^{109,110} Regenerative and proliferative capacities of growth factors after focal ischemic lesions were convincingly shown for basic fibroblast growth factor and osteogenic protein-1. Both compounds induced significant improvement of behavioral outcome without changes in infarct size when given 24 hours after ischemia.^{111,112} Growth factor treatment enhanced synaptogenesis and dendritic sprouting.¹¹¹ Basic fibroblast growth factor demonstrated a good safety profile in a phase 2 study, but phase 3 studies have been stopped.¹¹³

Citicoline

Citicoline (cytidine 5'-diphosphate choline), a naturally occurring endogenous compound that serves as an intermediate in the synthesis of membrane phosphatidylcholine, is thought to have membrane-stabilizing functions and to reduce free fatty acid formation during stroke. Citicoline reduces the size of infarction and improves neurologic outcome in experimental models of focal cerebral ischemia.^{114,115} In clinical studies, treatment with citicoline improved cognitive and behavioral function in patients with memory deficits.^{116,117} The drug was well tolerated in human studies without any known adverse effects.¹¹⁸ Therefore, citicoline was tested in a dose-response study at 0.5, 1, and 2 g beginning within 24 hours after stroke. The outcome based on the Barthel Index was significantly better only for the 0.5-g group.¹¹⁹ A randomized, double-blind, placebo-controlled phase 3 study of the 0.5-g dose of citicoline demonstrated negative results, although post hoc analysis indicated that medium and severe stroke patients benefited from the treatment.¹²⁰ A study targeted at patients with moderate-to-severe stroke was completed recently and did not show a significant effect on the primary outcome measure.¹²¹

Nootropic Agents

Piracetam is a γ -aminobutyric acid derivative and nootropic agent with neuroprotective capacities mediated through restoration of cell membrane fluidity and maintenance of membrane-bound cell functions. Experimental studies demonstrated a neuroprotective and regenerative effect of piracetam after focal lesions.¹²² However, a randomized, placebo-controlled IV trial, including 927 patients, produced negative results for mortality and outcome 12 weeks after stroke. Only a subgroup of patients treated within 7 hours showed a trend toward better neurologic outcome.¹²³ A new randomized, placebo-controlled, multicenter trial with a 7-hour time window has been started. Amphetamines increase release at the noradrenergic terminals of norepinephrine, dopamine, and serotonin

and could be future candidates for recovery studies after stroke. D-amphetamine improved behavioral outcome and memory function up to 60 days after focal cerebral ischemia. This was correlated with enhanced neocortical sprouting and synaptogenesis in the group treated with D-amphetamine.⁴⁷

Combination Treatment

Exploring effective combination therapies for stroke seems rational since cerebral ischemia triggers a multitude of pathophysiological and biochemical events that affect the evolution of focal ischemia differently. Impeding different steps in this cascade with different therapeutic agents may not only synergistically enhance the neuroprotective effect but may also allow the use of lower doses of each drug and consecutively less adverse effects. This approach demonstrated promise in several experimental studies and may serve as a future strategy for stroke therapy in humans. For example, combining low doses of citicoline with dizocilpine¹²⁴ or basic fibroblast growth factor¹²⁵ significantly reduces infarct size after focal cerebral ischemia, whereas a low dose of the compounds alone was not effective. Strategies include not only the combination of different neuroprotective agents but also the combination of thrombolysis and neuroprotection. Adding free radical scavengers such as tirilazad,¹²⁶ AMPA antagonists (NBQX),¹²⁷ NMDA antagonists (dizocilpine),¹²⁸ or citicoline¹²⁹ to rt-PA treatment, extends the time window and enhances the effect of thrombolysis after stroke. Combining agents for acute (eg, rt-PA, NMDA antagonists, free radical scavengers) and recovery treatment (eg, growth factors or citicoline) could be an important future approach to stroke therapy. However, more preclinical studies need to be completed before combination therapies should move into clinical development.

CONCLUSIONS

The current status of acute stroke therapy can be appropriately described as a combination of "the best

of times and the worst of times." The benefit of IV rt-PA when initiated within 3 hours of stroke onset documents that acute ischemic stroke can indeed respond to treatment. The results of PROACT-2 demonstrate that successful treatment can be extended to selected patients within 6 hours of stroke onset given an intra-arterial thrombolytic agent. This news is countered by the current lack of documentation that any purported neuroprotective drug significantly improves outcome when given after stroke onset. In addition, the approved use of IV rt-PA is restricted to the United States and Canada, where only a very small percentage of stroke patients receive this intervention. Many lessons have been learned from the myriad successful and unsuccessful thrombolytic and neuroprotective trials, suggesting that future, better designed trials will likely demonstrate significant benefits with appropriate safe and effective drug treatments initiated within 6 hours of stroke onset. Also, the age of combination drug trials is approaching rapidly and it is combination treatments directed at both the vascular and cellular mechanisms of ischemic brain injury that are likely to have the greatest impact upon stroke disability. This is not a time to abandon hope for developing safe and effective stroke therapies that are beneficial when initiated hours after onset. Rather it is a time to reflect on lessons learned from recent scientific advances and clinical trials to better move forward into the new millennium.

SUMMARY

The acute treatment of ischemic stroke to improve neurologic and functional outcome remains a challenging task with the potential of tremendous rewards for both patients and the health care delivery system. Currently, the only approved therapy is IV tissue-type plasminogen activator initiated within 3 hours of stroke onset in appropriately selected patients. Intra-arterial infusion of another thrombolytic agent, prourokinase, within 6 hours of stroke onset also improved outcome in a single trial, but the drug has not yet been approved for gen-

eral use. A large number of neuroprotective drugs were developed based on an enhanced comprehension of the mechanisms of focal ischemic brain injury. They were studied in pivotal clinical trials and so far there is no conclusive evidence of efficacy with any of these agents. It is hoped that effective neuroprotective therapy for acute ischemic stroke will be available soon and this therapeutic approach can then be combined with thrombolysis to enhance the benefits of acute stroke therapy.

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