Foreword

''Where tireless striving stretches its arms towards perfection. . .''

–Rabindranath Tagore

Clinical trials in emergency neurological disorders are not suited for the faint of heart. Most conditions under study have devastating consequences, the nervous system is not very forgiving of insults, the potential impact of any single therapy is limited, the therapeutic window of opportunity is generally brief and difficult to define, obtaining consent for participation can be a challenge, especially if the patient is cognitively impaired and unable to consent personally, patient accrual is usually limited at any single medical center, and uniformity of management is often elusive. Why then the interest?

Acute neurological disorders affect a large number of people in all age groups. The public health impact of these conditions therefore certainly cannot be ignored. It has been argued that the outcome from some of these diseases is determined *ab initio* and that any intervention is likely to be futile. However, the fact that the outcomes from many of the diseases have improved substantially over the past few decades argues against this nihilistic posture. It is fairly clear that improvements can, have and will be made. And the humanistic, intellectual and commercial pay-off of such advances is impossible to resist.

In this book Drs. Skolnick and Alves have brought together some of the most experienced investigators to create an invaluable road-map through this poorly charted and perilous territory. The result is a concise and easily digestible how-to manual that is a must-read for anyone who is in the field, or contemplating entry. Many lessons that have been learned the hard way from previous trials never make it into the published literature for a variety of reasons. Thus errors can be made over and over again. Therefore the greater value of this book may be in teaching us what not to do.

The editors have extensive real-world experience in the trenches of clinical trial design and implementation. The authors are leading experts in their respective disciplines. Together they have created a volume that investigators in the field of neurological emergency trials would ignore at their own peril. As we enjoy one of the most exciting eras in brain research, it is our hope that the information contained in this volume will serve as the foundation for many exciting breakthroughs in the not-so-distant future.

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sine qua non

WMA

This handbook was inspired by the dedication of the many clinician-scientists with whom I have had the opportunity to interact with and learn from over the past 20 years. Special acknowledgment to Howard I. Hurtig, M.D., and Mathew B. Stern, M.D. and my many other colleagues at the University of Pennsylvania who provided the foundations for my first explorations into the area of acute stroke in the early 80's. Finally, to my coworkers at Novo Nordisk who have enabled me to continue to apply these experiences in the CNS arena.

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BES

Introduction

Wayne M. Alves and Brett E. Skolnick

During the 1990s, scientific advances in understanding the mechanisms and pathophysiology of acute central nervous system injury, especially the neurochemical cascade associated with secondary brain injuries that occur most prominently with stroke and trauma, were offset by a history of disappointing results from phase III clinical trials of an unprecedented number of novel neuroprotective drugs. Novel compounds were ''tested'' and seemingly just fell by the wayside. The list of apparently ineffective compounds includes free radical scavengers, calcium channel blockers, and glutamate *N*-methyl-D-aspartate receptor antagonists along with many other classes of molecular targets. Were these disappointments reflective of failure of our therapeutic hypotheses or our inability to provide a level playing field to test the safety and efficacy of novel drugs?

The focus of this volume is the ''state of the practice'' of clinical trials in acute neuroscience populations, or ''neuroemergencies'' (1). Acute aspects of chronic neurological disorders, in so far as they pose special difficulties for evaluating novel therapies focused on the acute features of those diseases, are also relevant topics (e.g., drugs for acute exacerbations of multiple sclerosis or neuromuscular disorders). The book is intended to focus on novel therapies and the unique challenges their intended targets pose for the design and analysis of clinical trials.

We entered the 1990s as the clinical epidemiology of acute neuroemergencies was becoming well understood. High incidence, potentially devastating consequences, and recognition of the complexity of damage and outcome made these patients the sickest of the sick, with little or no effective treatments beyond supportive management and improved neurosurgical and neurointensive care management. This was combined with an unparalleled optimism regarding the potential of novel neuroprotective compounds. The Decade of the Brain provided disappointment as a legacy of failed clinical trials emerged. Table 1 lists some of the molecular and cellular targets for compounds that either failed or for which uncertain results

TABLE 1. Molecular and cellular targets of compounds in development for neuroemergencies since the 1990s

Neuroprotectants
Antioxidants/free radical scavengers
N-methyl-D-aspartate antagonists
Glycine antagonists
AMPA/kainite antagonists
Polyamine antagonists
Free fatty acid inhibitors
Adenosine antagonists
Bradykinin antagonists
Cholecystokinin B antagonists
Neurokinin receptor antagonists
γ-Aminobutyric acid agonists
Calcium channel blockers
Calcium-dependent protease (calpain) inhibitors
Sodium channel blockers
Lactate buffers/inhibitors
Nitric oxide synthase antagonists
Nonpsychotropic cannabinoids
Opiate receptor antagonists
Endothelin receptor antagonists
Apoptosis inhibitors
Gene expression regulators
Intracellular adhesion molecule inhibitors
Thrombolytics and antifibrinolytics
Recombinant tissue plasminogen activator
Streptokinase
Prourokinase
Fibrinogen-clearing enzyme
Tranexamic acid
Antifibrinolytic (e.g., Ancrod)
Anticoagulants and antiplatelets
Low-molecular-weight heparin
Heparinoids
Antiplatelet agents (e.g., Ticlopidine)

were obtained during the past 15 years. Although disappointments have been many, attempts to organize a consortium to handle the complexity of neuroemergency clinical trials offer hope (2).

Although neuroemergencies have a fairly high incidence, they are relatively rare compared with non–central nervous system diseases. They carry with them significant risk for devastating complications and long slow recovery. These are complex diseases and disorders with no singular recovery patterns. In some cases, similar injuries appear to have different outcomes, whereas in other cases the same outcomes result from quite different injuries. Morbidity is often underestimated, and factors of lifestyle and life cycle are important in both etiology and recovery. As such, not all the sequelae are directly attributable to injury *per se*, as indirect effects on important life domains are important and sociological factors contributing to outcomes lurk in the background.

The most significant emergent hypothesis of the 1990s regarding the potential of novel neuroprotective agents for neuroemergencies explicitly recognized that overlapping pathological processes in the early days postinsult led to irreversible cell damage or cell death, that early treatments were needed to interrupt a ''secondary cascade,'' and if successful we might observe improved cerebral metabolism with better clinical outcomes. The challenge was to find the ideal therapeutic milieu in which recovery could occur (3). It was left for us to test this hypothesis with new chemical entities with the potential to interrupt the secondary injury cascade.

By the mid-1990s over 100 new chemical entities were under development for a number of neurological disease indications, including about a dozen for traumatic brain injury. Yet we still have no approved drugs for traumatic brain injury, and only a single compound (recombinant tissue plasminogen activator) has been approved for use in ischemic stroke. This disappointing experience made it clear that safe and effective drugs would be hard to come by and success at best would be incremental. The problem, we are coming to understand, is how to find a level playing field to fairly demonstrate the safety, efficacy, and effectiveness of novel drugs targeted for neuroemergencies. Given that we have a need to recognize the multiplicity of damage and outcomes in clinical trials, the need to understand

factors that influence outcomes, and our past clinical trials failures, what do we have to offer?

The purpose of this volume is to explore the issues we face and the strategies that might lead to future success in developing drugs for neuroemergencies, which remains a critical area of unmet medical need. In retrospect, in our evaluation of past neuroemergency development programs, we are tempted to attribute our failures to skipping steps in the drug development process. This does not mean we should not strive to be creative in defining the ''optimal'' drug development paradigm for specific neuroemergency indications. The conventional drug development process is a staged sequential process that commercial scientists have long sought to reengineer and streamline. But the answer goes beyond simply the logistics of drug development. Our ability to define relevant treatment populations and measure the effects of treatment interventions is equally important. Improved disease classifications based on pathology and the use of continually improving imaging methods, improved endpoint measurement and analysis, identification of ''leveraged'' *in vivo* models to provide for better proof-of-concept studies, development of surrogate endpoints, and innovative clinical trials methodologies all can contribute to future success.

The minimal target criteria for a successful neuroprotectant are not difficult to describe. It must be safe, reach an intended action site (i.e., cross the blood–brain barrier), have an expected neurochemical effect, produce an expected neurophysiological effect leading to functional changes, and thereby improve clinical outcomes. The issue is how to demonstrate this in the conpara of adequate and well-controlled clinical trials.

Criticisms of previous neuroemergency development programs include bias in treatment group assignment due to imbalance in important covariates, inability to use classical statistical tests procedures, not addressing treatment delays, and difficulty in obtaining informed consent in many indications (see Chapter 13).

CURRENT STATUS OF TREATMENT OF NEUROEMERGENCIES

The brain is a small somewhat round object weighing approximately 3 pounds. As an organ, it has unique vulnerabilities. Its energy requirements demand a constant blood supply providing glucose and oxygen substrates. The brain is the organ most prone to spontaneous hemorrhage and second most prone to symptomatic ischemic infarction. Cerebral arteries are thinner and less elastic than in other systems of the body. Injury produces not only neurophysical impairments, but also changes in intellectual, emotional, and personality function (3).

Although the mechanisms of damage (e.g., infarction, hemorrhage, contusion, or edema) in neuroemergencies are limited, they seldom occur in isolation. It is often the case in the individual patient that several pathophysiological mechanisms are combined (1,2). This multiplicity of pathways for damage and outcome may be a major contributing reason for the failure of phase III clinical trials. The characteristic mechanisms of acute brain injury, listed below, are limited in that they tend to occur in combination with each other to create in each instance complexity of damage and outcome:

- Brain edema
- Hemorrhage
- Ischemia and brain swelling
- Hydrocephalus
- Neurotransmitter failure
- Toxic substances that cross blood–brain barrier
- Infection or inflammation
- Brain atrophy

Numerous reasons have been offered for the failure of clinical trials in acute neuroscience disorders in the 1990s, including whether the underlying therapeutic hypothesis is flawed, the nature of acute neuroscience populations, whether the drug is able to cross the blood–brain barrier, study design considerations, the clinical populations actually enrolled in the trials, and failure to control relevant disease cofactors. Especially relevant is the adequacy of brain penetration of the investigative agents tested in terms of optimizing dosage and the dosing regimens used. To address these issues and shortcomings, academic–industry collaboration has tried to define optimal preclinical and clinical strategies for drug development in ischemic stroke (6,7).

ACUTE NEUROCLINICAL TRIALS

There is a relatively limited history of drug development in acute neuroscience populations. As mentioned earlier, the diseases are fairly ''rare'' and require more research sites for sufficient enrollment. Individual practice variations in hospital-based settings (e.g., emergency departments or neurological intensive care units) contribute to a plethora of examinations, drugs, and supportive interventions. Treatment decisions are often idiosyncratic and there are few gold standards. Consequently, subjective definitions and perceptions are very important in guiding treatment decisions. Guideline statements are becoming more robust regarding treatment options but are still limited by the number of level I studies (8,9). This poses considerable challenges for the design, conduct, and analysis of randomized clinical trials. Because gold standards are few, often there is a lack of consensus on measurement of damage and outcome that contributes to large case report books. Because trials are large, they take time to conduct and analyze, and there is a danger that the rate of change in standards of clinical care could out-run our ability to prove efficacy. An example is the evolution of HHH therapy for the management of clinical vasospasm as a complication of subarachnoid hemorrhage as various pharmacological interventions were being tested. The fact that rescue therapies could have been efficacious (albeit risky and expensive) meant it was difficult to compare endpoints.

Many steps might be contemplated in improving neuroemergency trials, including:

- Identification of leveraged *in vivo* models that may reduce the inherent complexity of damage and outcome of neuroemergencies
- Improved efforts to understand underlying mechanisms of action
- Improved measurements of disease burden and/or activity
- Improved outcomes measurement
- Identifying procedures for handling the inherent overlap of various outcomes domains
- Identifying procedures for handling spillover and swamping effects of major prognostic factors
- Achieving agreement on how to order competing sets of explanatory variables in outcomes models
- Clinical phenotyping of treatment populations to avoid including patients with excessively good or excessively poor prognosis
- Focus on clinical benefit and crisper endpoint assessment
- Improved assessment of intermediate effects (i.e., biomarkers or mechanistic endpoints) as supportive evidence
- Consider ''novel'' approaches to neuroemergency trials design and randomization strategies.

PURPOSE OF THIS VOLUME

Modern clinical drug development involves complex interactions among scientific, medical, commercial, regulatory, and manufacturing issues (10). This volume is intended to provide developers of novel therapies with a more complete understanding of the scientific and medical issues of relevance in designing and initiating clinical development plans intended for acute neuroscience populations. We hope that we can provide an understanding of the pitfalls associated with drug development in neuroemergencies as well as a single source for the best information available regarding how to approach and solve the issues that have plagued drug development since the early 1990s.

We asked authors to include disorders generally requiring emergency care or intensive care in highly specialized clinical settings (e.g., neurological intensive care units). The authors could include discussion of drug development for disorders where the brain is a component (e.g., HIV-1 infection or sickle cell crises) and clinical development is primarily focused on brain protection in the setting of chronic disorders. Authors also could include neuroprotection in the compara of systemic disease (e.g., brain protection in coronary artery bypass graft surgery or out-of-hospital cardiac arrest). Device trials (e.g., endovascular obliteration of cerebral aneurysms) and brain access technologies where relevant could also be discussed. Out of sheer practicality, we excluded systemic complications in the compara of neuroemergencies (e.g., neurogenic cardiovascular disorders or respiratory syndromes), except as they are relevant to understanding the nature of the acute central nervous system disease and have implications for clinical drug development program. We also excluded evaluation of neurosurgical interventions *per se*, and drug development for disorders where the brain is a disease component but the therapeutic focus is on the systemic disease itself (e.g., HAART in HIV-1 infection as opposed to a drug focused on HIV-1–associated cognitive impairments).

The mandate to authors was to focus on relevant aspects of their respective disease areas that bore importance to the design and analysis of clinical trials. This could include the following:

- Brief overview of disease epidemiology and natural history
- Current management guidelines relevant for drug development
- Recent successes and disappointments of novel drugs
- Consensus regarding ''failed trials'' and how we might solve trials design and analysis problems
- Advances in preclinical evaluation of novel therapies
- Current ''state of the practice'' in the design and analysis of randomized clinical trials
- ''Gold'' and ''silver'' measures for diagnosis, definition of subpopulations, and outcomes assessment
- Biological markers and surrogate endpoints
- Emergent clinical technologies and methodologies relevant for future clinical trials (pros and cons). Examples include censoring excessively good or poor prognoses, shift analyses over a range of outcomes categories, and strategies for improving interrater reliability in outcome assessment.

No single volume can do justice to the complexity of drug development in acute neuroscience populations. Our hope is simply to stimulate discussion focused on providing solutions to the problems that have plagued the search for safe and efficacious drugs/biologics in the acute neurological area in the hope that investigators

will be able to provide the level playing field that has eluded us for so many years.

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CHAPTER

1

Acute Ischemic Stroke

Christopher Bladin and Stephen Davis

Stroke is one of the most devastating diseases of Western society. In most countries, stroke is the third most common cause of death and the leading cause of adult neurological disability (1). The social and psychological costs are enormous, and the health economic costs run into billions of dollars. Developing a successful and reliable acute treatment for stroke remains an elusive "Holy Grail." Fortunately, significant advances over the past decade indicate a breakthrough is not too far away.

STROKE THROMBOLYSIS

Intravenous tissue plasminogen activator (tPA) was approved for use in acute stroke in the United States in 1996 after publication of the landmark National Institute of Neurological Disorders and Stroke (NINDS) study (2). Approval for the use of tPA in acute stroke has occurred in many regions, including Canada (1999), Europe (2002), and Australia (2003). Acute stroke treatment guidelines, including use of tPA, have been published by a number of organizations, including the American Heart Association (3) and the Canadian Stroke Consortium (4). However, the benefits and risks of tPA in acute stroke are still the subject of much debate (5,6). Differences in trial methodology and outcome measures and conflicting results from various thrombolytic trials have made interpretation of the literature difficult and controversial for many. In addition, there have been claims of financial conflicts of interest in those devising these guidelines as well as concerns about inappropriate conclusions being drawn from the original NINDS publication. The *British Medical Journal* website has posted the many contributions to this often heated debate (5).

As a consequence, many neurologists and emergency medicine physicians have unfortunately expressed reluctance to use tPA in acute stroke. The knowledge base is therefore small, and only a few centers have depth of experience with stroke thrombolysis, further hindering the more widespread use of tPA. To fully understand the issues involved in the use of tPA in stroke, it is worth undertaking a brief overview of the seminal trials undertaken so far and following this with discussion on the phase IV (postmarketing) studies of tPA in acute stroke, otherwise known as "tPA use in the real world" (7).

Stroke tPA Thrombolysis Trials

As mentioned previously, the NINDS study was first published in 1995 (2). Acute

ischemic stroke patients were treated with tPA within 3 hours of symptom onset, and results indicated that those receiving this treatment achieved greater neurological recovery and experienced less disability than patients who received placebo. The tPA dose used was 0.9 mg/kg (maximum dose, 90 mg), and half of the patients were treated within 90 minutes of stroke onset. Patients in this study had moderately severe strokes with a median baseline score (National Institutes of Health Stroke Scale [NIHSS]) of 14 for tPA-treated patients and 15 for the placebo group. There was a strict protocol for managing hypertension, and all patients were admitted to the intensive care unit for the first 24 hours. Outcome measures were based on a ''global'' outcome score. This was a composite endpoint based on four disability scales (Barthel, Glasgow Outcome Scale, Rankin, and NIHSS) to detect a consistent and persuasive difference in the proportion of patients achieving a favorable outcome. At 3 months, each of the four primary outcome scales and the combined global tested statistics showed a statistically significant benefit for the use of tPA. In summary, 42% of the tPA-treated patients and only 26% of the placebo-treated patients had regained functional independence at 3 months. Overall, six patients (95% confidence interval, 5–11) had to be treated for one additional patient to recover self-care independence, and nine patients (95% confidence interval, 5–25) had to be treated for one additional patient to achieve full neurological recovery (7). The beneficial effects occurred in patients with all subtypes of stroke, including lacunar infarction. Further analysis of the NINDS data set revealed that the benefits were sustained at 1 year with no additional increase in mortality (6).

The occurrence of intracranial hemorrhage is the complication of most concern with tPA. These may be either asymptomatic (usually of small size) or larger symptomatic intracranial hemorrhages with clinical deterioration and possible impact on eventual outcome. In the NINDS study, symptomatic intracranial hemorrhages occurred in 6.4% of the tPA-treated patients and in 0.6% of placebo-treated patients ($p < 0.01$) (8). Most tPA-related hemorrhages occurred within the first 24 hours, and nearly half were fatal. The risk factors for developing intracerebral hemorrhage included increased stroke severity (NIHSS score) and hyperglycemia. Although the European tPA trials (9,10) suggested that baseline computed tomography (CT) findings of early cerebral edema with mass effect predicted hemorrhagic transformation with tPA, reanalysis of the NINDS trial did not suggest any major association (11). Despite the 10-fold difference in rate of symptomatic intracranial hemorrhage, the all-cause mortality rate was 17% for tPA-treated patients and 21% for placebo-treated patients (not statistically significant), with no increase in mortality attributable to tPA within the first week or even within the first 3 months.

Another argument that has been put forward is that some patients are ''rescued'' from death due to stroke only to be left with severe disability. However, the improved outcome in tPA-treated patients was not associated with an increase in the number of patients surviving with severe disability (2).

The NINDS tPA Controversy

The NINDS trial has undergone considerable scrutiny and interpretation since its publication (2). An imbalance in baseline stroke severity between the tPA and placebo treatment groups has been the primary focus of discussion (5,12,13) When the baseline NIHSS scores were divided into quintiles (0–5, 6–10, 11–15, 16–20, >20), it was found that imbalances existed in the mildest and most severe stroke groups. Of the 58 patients in the 0–5 NIHSS group, 42 (72%) were from the tPA treatment group, versus

16 (28%) from the placebo treatment group. Among the 140 patients in the >20 NIHSS group, 63 (45%) were from the tPA treatment group, versus 77 (55%) from the placebo treatment group. The imbalance in baseline stroke severity generated concerns that the treatment benefit reported in favor of tPA may have been explained by the excesses of both mild strokes allocated to tPA and more severe strokes allocated to placebo.

To determine whether the baseline stroke severity imbalance affected the outcome of the trial, the NINDS appointed an independent committee made up of three biostatisticians and three stroke clinicians to reanalyze the NINDS trial data. In addition to the issue of baseline stroke severity imbalance, the committee was asked to determine whether eligible stroke patients may not benefit from tPA given according to the protocol used in the trials. After performing extensive analyses, the committee reported that the baseline stroke severity imbalance did not affect the outcome of the study (14). Indeed, they confirmed on multivariate analysis evidence of a statistically significant tPA treatment effect. Exploratory analyses did not identify any group of acute ischemic stroke patients who would be harmed by receiving tPA. Specifically, there was no evidence that either baseline NIHSS or time from stroke onset to treatment modified the t-PA treatment effect.

Studies on tPA in acute stroke were also undertaken in Europe. The two studies performed were the European Cooperative Acute Stroke Studies, ECASS (9) and ECASS II (10). In the first ECASS study, the dose of tPA was higher than that used in the NINDS trial, at 1.1 mg/kg with a maximum dose of 100 mg. The other difference was that the window for administration of tPA was broader at 6 hours and the median time to treatment was 4 hours. There was a 21% incidence of intracranial hemorrhage in the tPA-treated patients. There were a number of possible causes for this, including the longer treatment window, the greater dose of tPA, and, perhaps most importantly, the inclusion of large numbers of patients (almost one in five) with protocol violations. These deviations mainly consisted of the failure to recognize changes on the pretreatment CT that should have excluded the patient from the study. As a consequence, there was no statistically significant difference in primary outcome where tPA-treated and placebo groups were based on the intention to treat analysis (9). In a reanalysis of the ECASS data (9), excluding patients who were inappropriately included in the study, the proportion of patients with minimal or no disability (modified Rankin scale of 0 or 1) at 3 months was significantly greater in the treatment group than in the control group (41% vs. 29%, $p < 0.05$).

With the many lessons learned during the first ECASS trial, ECASS II was undertaken in the late 1990s (10). The tPA dose was reduced to 0.9 mg/kg, as in the NINDS trial. Investigators were extensively trained to recognize the CT abnormalities of early ischemic stroke, in particular focusing on the exclusion of patients with more than one-third of the middle cerebral artery (MCA) territory involved in the ischemic process on the initial CT. Strict blood pressure controls were also implemented. The primary outcome measure was defined as the proportion of patients with a favorable outcome based on the modified Rankin scale score of 0 or 1 at 3 months, again in keeping with the NINDS trial. Based on this outcome measure, there was no significant difference between tPA treatment and placebo, although the distribution of modified Rankin Score (mRS) scores revealed a benefit in favor of tPA treatment. A post-hoc analysis was then undertaken, in which patient outcomes were dichotomized as either a good outcome, as indicated by independence in self-care (mRS score, 0 to 2), or

a bad outcome, as indicated by death or dependence (mRS score, 3 to 6). A significantly greater proportion of the tPA-treated patients achieved independence at 3 months (54% vs. 46%, $p = 0.024$) (10). From this post-hoc analysis it was determined that 12 patients had to be treated for one additional independent survivor. Intracranial hemorrhage was more common in the tPA-treated patients (9%) than in the placebo-treated patients (3%), but again there was no difference in mortality between the two groups.

One of the important points with ECASS II was that the time window remained at 6 hours, and the results indicated that tPA reduced disability without increasing the mortality rate. It should be emphasized that the primary outcome of the study was negative, and that a positive result was only achieved after a post-hoc analysis with reconfiguring of the methodological definition of ''favorable outcome.''

Another trial testing the effects of tPA on stroke was the Alteplase Thrombolysis for Acute Interventional Therapy in Ischemic Stroke study (15). This differed from other tPA studies in that it experienced a number of protocol changes due to publication of the NINDS trial and had two time windows: part A (<3 hours) and part B (3–5 hours). For part B there was no clinical benefit and there was an increase in mortality. Although the numbers in part A were small, there was a benefit in this cohort (16). The completion of four randomized controlled trials using tPA in acute ischemic stroke enabled several meta-analyses to be undertaken (17–19). These meta-analyses revealed an overall benefit for tPA treatment, regardless of the modified Rankin grading used to define outcomes of minimal disability/fully independent living, with no increase in mortality.

Guidelines for early management of acute ischemic stroke have now been extensively published and are freely available on the Internet (3,20). The recommendations are based on evidence-based practice and recommend the use of tPA in acute ischemic stroke in carefully selected patients, who can be treated within 3 hours of onset of ischemic stroke.

Phase IV Data: Postmarketing Studies in tPA Stroke Thrombolysis

There is now a large body of international experience in the use of tPA in acute ischemic stroke. Many centers have published data allowing perspective on the use of tPA in routine clinical practice (7).

The largest published experience is the Standard Treatment with Alteplase to Reverse Stroke study, which is a prospective review of the management of stroke with tPA in 24 academic and 33 community centers (21). The results from this phase IV study compared very favorably with those from the NINDS study. The characteristics of the patient population treated with tPA were similar to those in the NINDS study. The 1-month outcome indicated that 43% were independent and 35% had minimal or no disability. The rate of symptomatic intracranial hemorrhage was low at 3.3% compared with 6.4% in the NINDS study (21).

In Canada, after the approval for the use of tPA in acute stroke, a national database was required to be established as part of regulatory conditions for approval of tPA. The Canadian Activase for Stroke Effectiveness Study was established to collect data prospectively from academic and community hospitals across Canada (22). The results from this database indicated that patients receiving tPA were older than those in the NINDS cohort with a similar stroke severity. Again, the symptomatic intracranial hemorrhage rate was low (4.4%), and the 3-month outcomes were favorable and comparable with the NINDS data (7,22). Similar findings have been published from centers in Germany, the United States, and Australia (23–25).

An important message that came from the many postmarketing studies was the considerable danger in violating the tPA stroke protocol. This refers to treatment of patients who do not meet the eligibility criteria or are given additional treatments that deviate from published guidelines. The incidence of symptomatic intracranial hemorrhage in these patients is often much higher, with a greater risk of death or dependency. In one study from Indianapolis, tPA-related intracranial hemorrhage occurred in 38% of patients with protocol violations but only in 2% of those patients without protocol violations (26). A widely publicized report from Cleveland, largely based at community hospitals, highlighted the problems that can occur (27). In this cohort of 70 patients from 29 hospitals, the rate of symptomatic intracranial hemorrhage and in-hospital mortality was around 16%. This cohort had the highest reported rate of protocol violations, with 50% of patients deviating from national treatment guidelines. A quality improvement program with frequent educational sessions was then initiated to address these problems. A subsequent follow-up report in which stroke tPA guidelines were adhered to resulted in a significant improvement in outcome at the same group of hospitals (28).

Phase IV data should always be interpreted with caution, because there are often many differences between stroke centers (e.g., university hospitals vs. community hospitals), in patient demographics, in baseline stroke severity, and in the rate of protocol deviations. Adequate and complete follow-up, overall accuracy, and degree of completion of database sets are also very important.

Ongoing tPA Trials and Future Directions

There are still many challenges ahead for the proper administration of tPA in acute stroke. The poor public recognition of stroke symptoms, delays in transportation, and limitations of a 3-hour time window present considerable obstacles for patient recruitment (29). It is estimated that tPA treatment currently reaches only 2–3% of the North American stroke population (30). For example, in the NINDS trial, over 17,000 patients were screened, but only 624 eligible subjects were recruited. Most of those excluded were ineligible because of the time that had elapsed since stroke onset. Time delays from stroke onset to presentation continue to be frustrating. Public awareness of the symptoms of stroke is poor, and emphasizing the need to act quickly requires considerable education. Studies have shown that one-third of the general public cannot name a single warning sign of stroke (29). Prehospital stroke screening tools have been developed (e.g., the Los Angeles Pre-hospital Stroke Scale and the Cincinatti Pre-hospital Stroke Scale) to facilitate paramedic diagnosis of tPA-eligible stroke patients and to prenotify emergency departments of impending arrival (31,32). It is agreed that tPA should only be administered by physicians with expertise in acute stroke with strict adherence to published treatment guidelines. These may be neurologists or emergency medicine physicians with an interest in stroke. Similarly, nursing protocols, particularly for management of the post-tPA stroke patient, need to be closely followed. Comprehensive registries of tPA use, including hemorrhage rates, are used in Canada and Europe. Quality assurance via web-based database programs can simplify data collection and allow for more accurate postmarketing surveillance. Web-based programs have also been used to help improve skills in the radiological diagnosis of stroke on CT (e.g., www.neuroimage.co.uk) (33).

The target of acute stroke therapies, such as tPA, is the ischemic penumbra, a zone of incomplete ischemia where neurons are hypoxic and functionally inactive but still viable. The ischemic

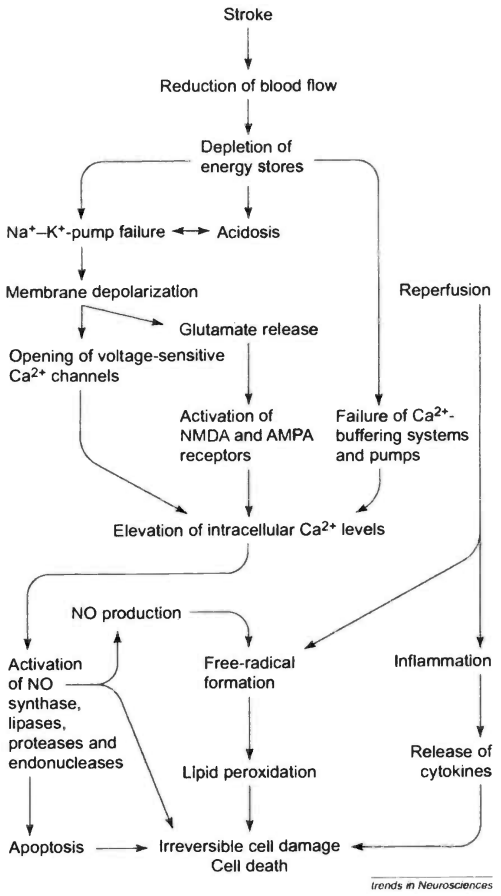

FIGURE 1.1 Neurotoxic cascade in the ischemic penumbra. A complex neurotoxic cascade is triggered by a focal deficit in brain perfusion. Key events are uncontrolled neuronal depolarizations, an overexcitation in glutamate receptors, a buildup of intracellular Ca^{2+} levels, the generation of free radicals, the stimulation of several catabolic systems, and the induction of inflammation. AMPA, ; NO, nitric oxide. (Adapted from ref. 44.)

penumbra is a dynamic time-based condition in which brain parenchyma undergoes necrosis over hours to days due to a cascade of biochemical events termed the ischemic cascade (Fig. 1.1). It has been suggested that the critical time for intervention, based on stroke studies using magnetic resonance imaging (MRI), may be around 4.5 hours, with earlier use of reperfusion strategies leading to greater tissue salvage (34). As the ischemic process unfolds, there is a progressive decrease in cerebral blood flow. When this falls from the normal levels of 50 to 55 ml/100 mg/min to below 8 to 10 ml/100 mg/min there is rapid neuronal cell death. However, between this ischemic core and the normally perfused brain at the periphery there exists zones of moderately reduced blood flow, the extent of which depends on collateral supply from surrounding arteries.

There are a number of ongoing trials to determine whether tPA can be used beyond the currently accepted 3-hour time window, targeting the penumbral region, which has been shown to last many hours in some patients (35). These include clinical trials such as ECASS 3 (tPA vs. placebo 3–4 hours after stroke onset) and the International Stroke Trial 3 (tPA vs. placebo 0–6 hours after onset). There has only been one intraarterial phase III trial of thrombolysis. The Prolyse in Acute Cerebral Thromboembolism trial used a 6-hour time window and focused on patients with MCA territory infarction, randomized to prourokinase versus placebo (36). The trial was positive, but another definitive trial is needed before this approach can be licensed.

There are also MRI-based trials, using combined perfusion-weighted imaging (PWI) and diffusion-weighted imaging (DWI), aimed at determining whether PWI–DWI mismatch can be used to select treatment responders beyond 3 hours. This MRI signature, where the PWI boundary represents tissue at risk and the DWI lesion represents tissue usually destined to infarct, is postulated to represent the ischemic penumbra, that is, critically hypoperfused but potentially recoverable brain tissue. These research trials include Echoplanar imaging thrombolysis evaluation trial (EPITHET) in Australasia and the Diffusion weighted imaging evoluation for understanding stroke evolution (DEFUSE) in the United States (34,37). The pilot EPITHET data suggested that tPA delivered to acute stroke patients with MRI

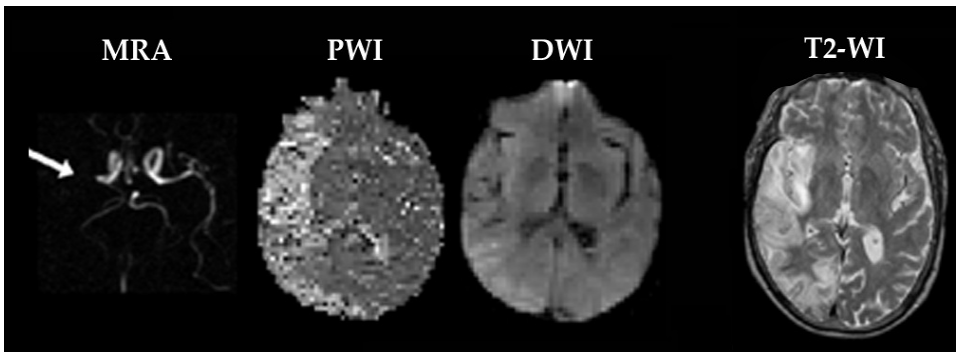

FIGURE 1.2 Acute right MCA infarct, with occlusion of proximal MCA on magnetic resonance angiography (MRA). Acute PWI–DWI mismatch is shown. In the absence of thrombolysis, an extensive infarct is present on outcome T2-weighted MRI (T2-WI). Rapid reperfusion has been shown to salvage tissue in the mismatch region.

mismatch enhanced brain tissue reperfusion and penumbral salvage (Fig. 1.2). Using this MRI-based approach, another thrombolytic agent, desmoteplase, has been tested 3–9 hours after stroke onset. Phase II studies have shown enhanced reperfusion to the ischemic region, very few intracerebral hemorrhages, and promising outcome data. Phase III studies will follow (38).

ANTITHROMBOTIC DRUGS

Acute aspirin is now used almost routinely in acute ischemic stroke, based on the two megatrials, the International Stroke Trial and Chinese acute stroke trial (CAST), which showed that aspirin given within 48 hours modestly reduced poor outcomes at 6 months (39–41). The exception is for patients who have received tPA, where antiplatelet therapy is contraindicated for 24 hours after tPA administration. These trials suggested that for every 1000 patients treated, poor outcomes could be reduced in about 10 patients. There is controversy whether this is truly an acute benefit or in fact a secondary prevention effect. Another acute antiplatelet approach, using the intravenous glycoprotein 2b/3a antagonist abciximab, has yielded promising results in a phase II trial and is being studied further (42).

In contrast, formal anticoagulation with heparin or low-molecular-weight heparin or heparinoids has not been beneficial in acute stroke, In most trials, a small reduction in acute recurrent stroke has been offset by a greater increase in rate of intracerebral hemorrhage (40,41,43). These agents are still sometimes recommended in patients with minor ischemic stroke and a very high risk of recurrent embolism (e.g., prosthetic valve disease). They are also used in cerebral vein thrombosis, extracranial arterial dissection, and in low dose for deep vein thrombosis prophylaxis.

NEUROPROTECTIVE DRUGS IN ACUTE ISCHEMIC STROKE

The use of tPA in acute ischemic stroke realistically represents only one part of the treatment equation. Apart from restoring blood flow to ischemic brain tissue, the cellular and pathobiochemical consequences of prolonged cerebral ischemia also need to be dealt with. In the ischemic penumbra, oxygen delivery becomes insufficient to allow normal levels of oxidative metabolism. This produces lactic acidosis and impedes the production of ATP, the energy source of cellular ionic pumps.

Failure of the sodium-potassium pump results in rapid loss of potassium from the neurons with extensive neuronal depolarization. Voltage calcium channels are opened, leading to an extracellular build-up of excitatory amino acids that overstimulates receptors. One of the principal excitatory amino acids is glutamate, with stimulation of the *N*-methyl-D-aspartate (NMDA) and AMPA receptors, a key component of the excitotoxic process. There is failure of the mitochondrial energy systems, resulting in elevated calcium levels, the generation of free radicals, and formation of excessive amounts of nitric oxide. Lipid peroxidation occurs, resulting in membrane damage, and there is sustained activation a large range of calcium-dependent enzymes (e.g., lipases, proteases, endonucleases, etc.) with further impairment of cellular function and membrane structure. As a background to this there is also damage to cellular DNA via endonucleases or free radicals. This triggers a complex self-destructive process involving gene expression, known as apoptosis, or programmed cell death. Mitochondrial dysfunction is thought to be a key element in the initiation of apoptosis with release of caspases, cytochrome c, and mitochondrial apoptosis-inducing factor (44).

The restoration of blood flow, for example via thrombolysis, may potentially exacerbate this process. Oxygen can enhance the biochemical reactions that generate free radicals. Inflammatory processes also play a roll with up-regulation of endothelial adhesion receptors and other chemoattractants resulting in invasion of leucocytes and macrophages and the release of metalloproteinases and cytotoxic cytokines (such as tumor necrosis factor and interleukins).

Potential Targets for Neuroprotection

The many stages of the neurotoxic pathway outlined in Figure 1.1 would seem to offer a wealth of opportunities to impede or alter the course of this dynamic process. In animal studies, reduction of infarct size of 50% or even greater has been demonstrated with strategies that attenuate the excitotoxic cascade, reduce free radical toxicity, diminish the various inflammatory responses, and finally curb progressive neuronal cell death by apoptosis (44–46).

Neuroprotective agents can target one or more of these processes. Following are some examples of areas that have been pharmacologically targeted and the results of trials that have ensued.

1. Sodium channel blockers: The anti-epileptic medication phenytoin blocks voltage-dependent sodium channels and reduces infarct size in permanent and reperfusion models of focal brain ischemia (47). Fosphenytoin is a prodrug of phenytoin that was evaluated in a phase III trial; enrollment was halted because of lack of efficacy in an interim analysis (48).
2. Calcium channel blockers: The prominent roll of calcium in the excitotoxic process has led to many attempts to develop therapies to inhibit voltage-sensitive calcium channels. Nimodipine and flunarizine are calcium channel blockers that have been shown to reduce infarct size in animal models of permanent and transient focal cerebral ischaemia (49). Nimodipine has been demonstrated to be of significant benefit in subarachnoid hemorrhage but has been less than impressive in trials for acute ischemic stroke, with worsened outcome when administrated intravenously. One particular problem was the marked hypotensive effect of intravenous administration. A meta-analysis of studies using oral nimodipine with a broad 12-hour time window suggested a possible benefit for the drug. However, the prospective, randomized, controlled trial, VENUS (Very Early Nimodipine

Use in Stroke), was stopped due to a failure of benefit (50).

3. Glutamate inhibition: Blockade of the NMDA receptor was an early target in the development of neuroprotective agents with promising data from animal studies. Early studies of various NMDA receptors were stopped in phase I and II development because of unacceptable neuropsychiatric side effects (51). Some agents (e.g., selfotel, aptiganel, eliprodil) were studied in phase III trials but were terminated prematurely because of poor benefit (44,52–54). Selfotel was thought to have neurotoxic effects, with increased early mortality (52).
4. γ-Aminobutyric acid (GABA) agonists: The use of drugs that cause activation of GABA receptors have been proposed as a strategy to counteract the actions of glutamate. Clomethiazole is an antiepileptic drug that causes neuronal hyperpolarization by enhancing the activity of GABA and $GABA_A$ receptors (44). Again, different ischemic animal stroke models indicated a neuroprotective effect for clomethiazole, but phase III clinical trials failed to show any benefit (55). A post-hoc analysis did indicate that patients with severe stroke may receive some benefit, leading to the establishment of the Clomethiazole Acute Stroke Study Ischemia. This study concluded with a negative result (56).
5. Nitrogen oxide inhibitors and free radical scavengers: The rise in intracellular calcium levels in ischemia leads to activation of nitric oxide synthase and nitric oxide production, a cytotoxic free radical. Down-regulation of the nitric oxide synthase pathway has been investigated using drugs such as lubeluzole, a purported neuroprotective agent that acts by reducing nitric oxide-related neurotoxicity. Initial animal stroke studies and small low-dose phase II human studies indicated reduced mortality in ischemic stroke (44,57). However, a number of phase III trials, some using a double-dose regimen producing plasma concentration equivalent to the levels associated with neuroprotection in rats, failed to produce any benefit in over 3000 patients (57,58).

   Free radical scavengers have also been investigated. Tirilazad is a nonglucocorticoid lipid peroxidation inhibitor that acts as a free radical scavenger. As with many other neuroprotective agents, this drug demonstrated reduced cerebral infarct volume in animal models but in phase III studies in humans did not result in any improvement in functional outcome (59). Concerns were then raised about the dose being inappropriately low, but subsequent trials with higher doses of tirilazad were stopped prematurely because of safety problems (60).
6. Anti-inflammatory agents: The inflammatory process is an integral part of neurotoxicity in acute ischemic stroke. Adhesion molecules such as endothelial adhesion molecule-1 are rapidly expressed in the zone of focal cerebral ischemia attracting leucocytes into the region of ischemia and with cytokine release further enhance developing necrosis. Enlimomab, a mouse monoclonal antibody against endothelial adhesion molecule-1, was demonstrated to be effective in animal models in reducing infarct volume in reperfusion models but not with permanent MCA occlusion models (61,62). Phase III studies indicated that there is indeed a neurological deterioration in patients receiving this treatment, quite possibly related to the mouse antigens provoking an inflammatory response (44).

   Other agents that have been studied include those acting at the cell membrane level such as G_{M1}-ganglioside, citicoline, and piracetam (63–65). As with other agents, results in phase III trials have largely been disappointing.

G_{M1}-ganglioside licenses have been suspended due to concerns over possible occurrence of Guillain-Barré neuropathy.

STUMBLING FROM THE BENCH TO BEDSIDE

Despite extensive research with many different compounds, which have demonstrated promising results in animal stroke models, all phase III clinical trials conducted so far indicate that these drugs have failed to live up to their initial promise (46,51,52,66,67). Many compounds that interfere with the excitotoxic pathway have been demonstrated to be neuroprotective in preclinical models of stroke. Safety in subsequent phase I and II clinical trials led to phase III, randomized, double-blind, placebo-controlled, efficacy trials. The resources required to complete such a trial are prodigious, often estimated to be over 50 million U.S. dollars. Despite these efforts, all phase III trials have so far failed to demonstrate the efficacy of neuroprotective agents. The reader looking for further detailed analysis on the many trials conducted in this area is directed to a number of excellent published reviews and commentaries (44–46).

Failure of Clinical Trials in Neuroprotective Therapies

The common theme in all trials of neuroprotective therapies is the failure of promising results in animal models of ischemic stroke to be replicated in phase III clinical trials. There are many possible reasons for this (44,54,68), including difficulty in translating tightly structured animal stroke models to complex human clinical scenarios and inadequate penetration of neuroprotective agents into the poorly perfused hemisphere and ischemic penumbra. It has also been suggested that the secondary and reperfusion biochemical cascade may be responsible for relatively little additional tissue damage compared with that due to the initial hypoxic ischemic insult (69). Other possibilities include the relatively long time windows used in many neuroprotective trials, adverse effects of some drugs such as hypotension and sedation, inadequate sample size, other trial design issues, and the relative lack of neuroreceptors in white matter.

Stroke Heterogeneity

Stroke in the human brain is much less predictable than in the animal model: The etiology, location, and severity of ischemic stroke in human subjects is very heterogenous. All ischemic stroke animal models are based around permanent or reversible models of ischemia. In reversible cerebral ischemia the vessel is occluded, usually with a silicon or cotton thread for variable time periods ranging from several minutes to several hours. The occlusive device is removed and cerebral perfusion is allowed to reoccur. Laser Doppler flowmetry is now considered an essential part of all animal studies to ensure that reperfusion has indeed occurred. In permanent ischemia models, the occlusion is left *in situ* with no reperfusion allowed to occur. A number of neuroprotective agents have demonstrated efficacy in one of these ischemic models but not in the other.

However, the animal model is set in a highly contrived and stabilized environment using animals of similar age and standardized amounts of focal cerebral ischemia induced by a reproducible intervention. In humans, both types of focal brain ischemia can, and often do, occur, producing a potentially salvageable ischemic penumbra; reperfusion after transient occlusion further adds to the evolving neurotoxicity. Collateral circulation influences infarct and penumbral size (70), with considerable variability in the human

model of collateral flow around the circle of Willis that is often only partially complete. Variability in the location of the embolic occlusion (e.g., proximal or distal MCA) also leads to variability in the ability of the collateral branches, such as those through the lenticulostriate arteries, to sustain cerebral parenchymal blood flow.

The human stroke model has patients of different ages with variable comorbidities, all of which can heavily affect the outcome of death or disability. The nature of ischemic stroke is also variable, ranging from large cortical infarctions through to small lacunar infarction. Variations in collateral circulation may further alter the size of the ischemic penumbra. Many patients show spontaneous reperfusion in the early stages of stroke and consequently have a better clinical outcome. At the time of stroke, systemic factors such as blood pressure, body temperature, oxygenation, and glucose levels can also affect eventual outcome and can potentially override any beneficial effect of a neuroprotective agent (see later).

Optimal Therapeutic Doses of Neuroprotective Agents

Determining the correct balance between the adverse and beneficial effects of neuroprotective drugs is often difficult. In many studies, doses of neuroprotective drugs that limit infarct size in animals are associated with adverse effects that can limit tolerable doses. Psychiatric side effects were the main reason for the premature termination of a number of trials with NMDA receptor antagonists, and adverse hemodynamic consequences have limited the efficacy of drugs such as nimodipine (71). Conversely, suboptimal doses of neuroprotective agents may be used in phase III trials because of undue concern regarding some safety aspects. In phase III trials of lubeluzole, concerns about the QTc interval were possibly misinterpreted from the phase II trial data, leading to an incorrect dosage regimen (58,72). In other examples, the length of administration of neuroprotective agent may not be sufficient; clomethiazole was administered for only 24 hours, although it had been demonstrated that excitatory amino acid levels in the ischemic penumbra could remain significantly elevated for at least 6 days after the onset of stroke. Concerns of excessive sedation shortened the protocol for administration of this medication to a probable inappropriate time period.

Combination Neuroprotective Therapies

Developing therapies that target the hypoxic brain cell is only the first step; appropriate delivery to this site is clearly required and, in many ways, can only be done after vascular reperfusion has occurred. Combination with a thrombolytic drug is the next natural progression to this process, at which point the combination of a ''clot buster and a cell saver'' is really more likely to succeed.

Most, if not all, neuroprotective drugs developed so far target one specific aspect of the ischemic cascade pathway. To expect a definitive result from this limited approach is in many ways unrealistic, and a multimodal therapy, targeting multiple areas of the ischemic cascade, would be more practical and likely to succeed. An example of this is the novel agent AM-36, an arylalkylpiperazine with combined antioxidant and Na^+ channel blocking actions (73). Individually, these properties have been shown to confer neuroprotection in a variety of *in vitro* and *in vivo* animal models of stroke. Preliminary studies have demonstrated that AM-36 is neuroprotective *in vivo* and protects against both neuronal damage and functional deficits even when administered up to 180 minutes after induction of stroke. In fact, the greatest protection was found when administration was delayed by 180 minutes after stroke (73). This

multimodal approach to neuroprotective therapy clearly holds great promise.

Physiological Modification of the Ischemic Environment

There is increasing interest in physiological approaches that impact on the acute ischemic process. These strategies include careful maintenance of euglycemia in hyperglycemic stroke patients, aggressive treatment of fever, varied ways of inducing hypothermia, and potential manipulation of blood pressure in the acute setting.

About one-third of acute stroke patients present with hyperglycemia. These include known and newly diagnosed diabetic subjects, but also those with stress hyperglycemia. These groups can be distinguished by measurements of acute blood glucose and HbA1C to determine whether the hyperglycemia has predated the stroke. Regardless of etiology, hyperglycemia independently predicts higher mortality and worse functional outcome (74). Although the precise mechanism is unclear, higher lactate levels have been identified in animal and human studies in the ischemic region, consequent upon activation of the glycolytic pathway in hyperglycemia in anaerobic regions (75). A large phase III trial is investigating whether dextrose-insulin infusions improve outcome (76). In the meantime, stroke guidelines currently advocate avoidance of glucose-containing solutions in acute stroke and correction of hyperglycemia.

In both animal and human stroke, fever is independently associated with a worse outcome (77). Fever is known to accentuate the neurotoxic cascade after acute ischemic stroke, as demonstrated in animal models. There is a consensus that fever should therefore be aggressively treated. Furthermore, small phase II studies indicated that mild to moderate hypothermia, aiming at a core temperature of about 33°C, might be beneficial (78). Techniques to induce hypothermia include external cooling blankets, cooled intravenous fluids, and intravenous heat-exchange catheters that can rapidly and precisely induce and maintain hypothermia and subsequent rewarming. Larger trials are planned.

In ischemic brain, perfusion pressure is important, but the normal autoregulation (where cerebral blood flow is maintained constant despite wide fluctuations in blood pressure) is lost in acute stroke. There is enormous uncertainty about the optimal approach to blood pressure management in acute stroke, and this remains a great challenge in stroke trials. Strategies have ranged from pressor therapies to elevate perfusion pressure to a variety of blood pressure–lowering therapies (79). It is recognized that marked acute hypertension increases the risk of hemorrhagic transformation in ischemic stroke treated with tPA, so that there is an recommended upper limit set of 180/100 mm Hg. Conversely, rapid blood pressure lowering is generally considered hazardous. Elevated blood pressure levels in acute stroke tend to spontaneously lower over the first 24–48 hours. Current trials include the use of glyceryl trinitrate (GTN) paste to lower blood pressure, being tested in efficacy of nitric oxide in stroke (ENOS) (80).

FUTURE STROKE TRIALS: THE STROKE THERAPY ACADEMIC INDUSTRY ROUNDTABLE

The difficulty with current animal models and the poor rate of progression from phase II to phase III studies, coupled with the failure of many phase III studies, led to the development of the Stroke Therapy Academic Industry Roundtable (STAIR) (68). This collaborative group is actively examining preclinical issues and trial design to ensure the optimal development of new acute stroke therapies. There has been particular scrutiny of phase II and phase III studies with

regard to trial design and appropriately valid outcome measures/endpoints that are easy to measure, reproducible, valid, clinically meaningful, and resistant to bias (68).

Stroke is a heterogenous entity, and a ''one size fits all'' approach to treatment is not appropriate. The challenge is to reliably identify the different stroke subgroups and tailor the therapy accordingly. Experimental data in animal stroke models suggest that the treatment window may be as long as 8 to 12 hours. Imaging and biochemical studies in acute stroke patients suggest that a similar time window may be present in selected stroke patients. The STAIR group is examining the incorporation of imaging methods into trial methodology. Imaging techniques such as multimodal MRI and CT using diffusion and perfusion imaging offer an opportunity to greatly assist patient selection (68,81) and may allow the expansion of the therapeutic time window beyond 3 hours, with some trials investigating treatment up to 9 hours (82).

CONCLUSIONS

Treatment of stroke in the new millennium offers much promise, with a renewed interest and vigor for the management of acute ischemic stroke with both clinical therapies and pharmacotherapies. Yet despite all this good work, one of the key difficulties still remains targeting the acute stroke patient as quickly as possible to administer these treatments. Our excellent stroke research will have only minimal public health impact if only a small percentage of the stroke population is able to receive and benefit from these new therapies. Education is an integral part of this process, both to our colleagues and to the general public. In many ways, this represents equally as great a challenge to stroke physicians and stroke researchers.

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