Toward Wisdom From Failure

Lessons From Neuroprotective Stroke Trials and New Therapeutic Directions

David J. Gladstone, BSc, MD; Sandra E. Black, MD, FRCPC; Antoine M. Hakim, MD, PhD, FRCPC; for the Heart and Stroke Foundation of Ontario Centre of Excellence in Stroke Recovery

Background—Neuroprotective drugs for acute stroke have appeared to work in animals, only to fail when tested in humans. With the failure of so many clinical trials, the future of neuroprotective drug development is in jeopardy. Current hypotheses and methodologies must continue to be reevaluated, and new strategies need to be explored.

Summary of Review—In part 1, we review key challenges and complexities in translational stroke research by focusing on the "disconnect" in the way that neuroprotective agents have traditionally been assessed in clinical trials compared with animal models. In preclinical studies, determination of neuroprotection has relied heavily on assessment of infarct volume measurements (instead of functional outcomes), short-term (instead of long-term) end points, transient (instead of permanent) ischemia models, short (instead of extended) time windows for drug administration, and protection of cerebral gray matter (instead of both gray and white matter). Clinical trials have often been limited by inappropriately long time windows, insufficient statistical power, insensitive outcome measures, inclusion of protocol violators, failure to target specific stroke subtypes, and failure to target the ischemic penumbra. In part 2, we explore new concepts in ischemic pathophysiology that should encourage us also to think beyond the hyperacute phase of ischemia and consider the design of trials that use multiagent therapy and exploit the capacity of the brain for neuroplasticity and repair.

Conclusions—By recognizing the strengths and limitations of animal models of stroke and the shortcomings of previous clinical trials, we hope to move translational research forward for the development of new therapies for the acute and subacute stages after stroke. **(*Stroke*. 2002;33:2123-2136.)**

Key Words: cerebral ischemia ■ clinical trials ■ models, animal ■ neuroprotection ■ rehabilitation ■ stroke ■ translations

Neuroprotection for Stroke: A Fantasy Invented by Basic Scientists?

The quest for effective stroke treatments remains an urgent priority. A stroke occurs every 53 seconds in North America,[1] and by 2020, cerebrovascular disease is projected to become the fourth-leading burden of disease worldwide, after heart disease, depression, and motor vehicle collisions.[2] According to the summary of historical trends in clinical stroke trials by Kidwell et al,[3] the 20th century saw the publication of 178 controlled trials of acute stroke therapies in the English-language literature, yet only a few produced "positive" results: the Neurological Institute of Neurological Disorders and Stroke (NINDS) rt-PA trial,[4] Prolyse in Acute Cerebral Thromboembolism (PROACT II),[5] a low-molecular-weight heparin trial,[6] and most recently a trial of ancrod.[7] The successful translation of these "vascular approaches" from the animal laboratory to the hospital emergency room has demonstrated that stroke is a treatable disorder in the hyperacute stage and has provided optimism that additional therapies to improve stroke outcome will be possible in the future.

In contrast, neuroprotective drugs that aim to salvage ischemic tissue, limit infarct size, prolong the time window for reperfusion therapy, or minimize postischemic reperfusion injury or inflammation have shown great promise in preclinical testing but disappointment in clinical trials.[8–10] Of >49 neuroprotective agents studied in >114 stroke trials, none has proven successful clinically.[3] Similarly, neuroprotective therapy has been unsuccessful in clinical trials of head trauma.[11–14] With the failure of so many trials, some clinicians may ask, "Is neuroprotective stroke therapy just a fantasy invented by basic scientists?" Will it ever play a clinical role? The answer is unclear. It must be acknowledged that neuroprotection may never be effective for promoting functional recovery after brain injury in humans. However, a lack of evidence of efficacy does not necessarily mean a lack

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From the Division of Neurology and Regional Stroke Program, Sunnybrook and Women's College Health Sciences Centre, and Institute of Medical Sciences (D.J.G., S.E.B.), University of Toronto, Toronto, Canada, and Neuroscience Research, Ottawa Health Research Institute, University of Ottawa, Ottawa, Canada, and Canadian Stroke Network (A.M.H.).

Correspondence to Dr David Gladstone, Cognitive Neurology and Stroke Research Unit, Room A421, Sunnybrook and Women's College Health Sciences Centre, 2075 Bayview Ave, Toronto, Ontario, Canada M4P 3 M5. E-mail david.gladstone@utoronto.ca

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of efficacy. Because of problems in basic science experiments and in clinical trial design, the evidence against neuroprotection is not conclusive, as is discussed below.

The purpose of this article is to provide clinicians and investigators with an up-to-date summary of the complex issues involved in translating stroke-related research from bench to bedside and to argue that experience from recent trials can provide important lessons that can be translated from the bedside back to the bench. Part 1 reviews pitfalls that have arisen in the development of neuroprotective therapies and reinforces recent recommendations regarding preclinical and clinical evaluation of new drugs.[15,16] Part 2 highlights emerging concepts in ischemic pathophysiology that should encourage us to think beyond the hyperacute phase of ischemia and consider the design of trials that use multiagent therapy and exploit the capacity of the brain for neuroplasticity and repair.

Part 1: Reconciling the Results of Negative Stroke Trials

Reasons for the failure of so many neuroprotective agents in clinical trials, despite their apparent benefit in animal (mostly rodent) models, have been the subject of intense discussion recently.[15–31] It is becoming clear that existing animal models of focal cerebral ischemia are an imperfect representation of human stroke and may be relevant only to a minority of human stroke types.[32] Neff[33] reminds us that the lissencephalic brain of a rat is about the size of a lacunar infarct in humans, and some humans have infarcts the size of an entire rat. In addition, despite significant similarities between the rodent and human genomes, the differences that do exist are sufficient to remind us that conclusions reached regarding genomic and proteomic characteristics in rodent studies may not apply to human stroke.

Neuroanatomical, pathophysiological, pharmacokinetic, and genetic differences between rodents and humans notwithstanding, there has been a fundamental "disconnect" in the way that the efficacy of putative neuroprotective agents has been assessed in animal studies compared with clinical trials. The dramatic rise in the number of stroke trials over the past several decades has been accompanied by an improving, yet still highly variable, quality in study design.[3,34] Some trials could not have been expected to succeed because of conceptual or methodological flaws. Perhaps we have abandoned efficacious treatments prematurely on the basis of results of flawed trials. Other trials have been criticized, in retrospect, for proceeding on the basis of insufficient evidence of efficacy in preclinical studies (eg, only 50% of published animal studies were in favor of nimodipine).[35] Other studies, still, have raised safety concerns because of drug toxicity, a danger of accelerating research by combining phase II and phase III trials.[36–38]

In this section, we highlight 9 pitfalls that have arisen in trying to extrapolate from animals to humans in the investigation of neuroprotective therapy. By understanding how animal models may be made more relevant to human stroke and how the design of clinical trials may be improved, we can move forward translational research for the development of stroke therapies.

Pitfall 1: Preclinical Studies Have Used Very Short Time Windows for Drug Administration, Whereas Clinical Trials Allow Longer Time Windows

Most neuroprotective studies in animals have relied on drug administration either before the ischemic insult or very soon after the onset of ischemia.[39,40] In contrast, time windows for entry in acute stroke neuroprotective trials have been longer and highly variable; in studies published between 1995 and 1999, the median time to entry was12 hours (range, 4 hours to 12 days), with a median time to treatment of 14 hours.[3] None of the published neuroprotective trials has used a 3-hour window.[3,16] Treatment within 3 hours would be expected to have a greater chance of efficacy because more patients would be expected to have potentially reversible ischemic tissue.[40–42] The effects of neuroprotective agents in the laboratory are even more time dependent than thrombolytics, leading Jonas et al[19] to summarize the failure of neuroprotective trials as a matter of "too little, too late." Certain "failed" drugs could potentially have clinical value if given at earlier time periods (within 2 hours after ischemia or prophylactically).[19]

On the other hand, Baron et al[43,44] and Fisher et al[45] emphasize that we need not stipulate a fixed time limit for neuroprotective therapy because the duration of the ischemic penumbra is highly individualized. For example, PET studies suggest that the window of opportunity may be extended in some patients[46]; in 1 study, about one third of patients still had evidence of penumbra when assessed at 5 to 18 hours (mean, 10 hours) after stroke onset.[44] The PROACT II results further support the fact that salvageable tissue is present up to 6 hours after onset in some patients.[5] The rate of progression of the penumbra from reversible to irreversible ischemic injury is dependent on many factors and may be accelerated in the presence of poor collateral circulation, hyperglycemia, and other exacerbating factors.[45]

Putative neuroprotective drugs should not be advanced into clinical stroke trials until preclinical studies have investigated their effects when administered many hours, not minutes, after ischemia. Clinical trialists must aim for the shortest possible door-to-needle times, particularly given the tendency that physicians have of "waiting until the last minute" of the time window to treat, regardless of when the patient arrives at hospital.[47] The NINDS rt-PA study showed that enrollment within 3 hours can be achieved, although 17 324 patients were screened to recruit the 624 subjects eligible for the study, with most excluded because of the time window.[4] With increasing public awareness of this issue and improvements in regional organization of stroke services and stroke teams, response times are improving, and at some centers, >25% of patients are reaching hospital within 3 hours.[48] Clinical trial protocols should enforce benchmarks for door-to-needle times and stratify patients by time of treatment with appropriate power calculations. If the long time windows are a major reason for the lack of efficacy of neuroprotective therapy in human trials, then the investigation of agents given prophylactically (eg, before surgical procedures with an increased risk of ischemic cerebrovascular events)[49–52] or by paramedics in the field (a phase I trial is already underway) may provide the necessary "proof-of-principle" data that are

much needed as long as a strict target population can be defined by preplanned posthoc analysis.

Pitfall 2: Preclinical Studies Target the Ischemic Penumbra, Whereas Clinical Trials Do Not

As Fisher,[45] Baron,[53] and others[54] have emphasized, the target of current neuroprotective therapy is the penumbra, ischemic tissue that is functionally impaired but whose damage is potentially reversible.[55,56] If reversible ischemic tissue is not present at the time of treatment, then neuroprotective therapy cannot be expected to work. Perhaps we have discarded some agents prematurely because clinical trials have not been selective enough in targeting patients with evidence of penumbra. Future trials may need to use stricter entry criteria to target not just cortical strokes but specifically those with a sufficient volume of penumbra.[16,45]

Patients with potentially salvageable penumbra tissue may be identified by functional neuroimaging.[44,45,53,57–59] According to PET studies by Heiss et al[57] performed in patients within 3 hours of acute stroke, the penumbra made up 18% (range, 8% to 34%) of the final infarct volume; 70% (range, 51% to 92%) was already critically hypoperfused, and 12% (2 to 25%) was sufficiently perfused. Although such observations imply that on average neuroprotective therapy may be able to salvage only a relatively small fraction of an infarct (supporting the rationale for combination reperfusion-neuroprotective studies), some patients can be identified with larger volumes of penumbra.[46,60] A recent PET study suggested that 45% of the final infarct (and in some patients, up to 85%) remained viable for up to 12 hours.[60,61]

With MRI, a perfusion-diffusion mismatch (perfusion abnormality greater than diffusion abnormality) can be identified in ≈70% of patients studied within 6 hours of stroke onset and may indicate salvageable tissue.[62,63] Some trials are now using MRI criteria to improve patient selection[64] (eg, a trial of sipatrigine currently underway requires a perfusion-diffusion mismatch at baseline of at least 30%[65]). Dynamic CT perfusion imaging also provides promise as a method of acute stroke imaging that may allow rapid identification of tissue compartments perfused within predetermined blood flow thresholds.[66]

In the absence of perfusion imaging, a mismatch between the clinical deficit and imaging findings has been suggested as a way to optimize patient selection (ie, severe clinical deficit with limited early lesion on diffusion-weighted MRI or CT).[54] A prediction formula that incorporates the time elapsed, National Institutes of Health Stroke Scale (NIHSS) score, and diffusion-weighted MRI lesion has recently been validated.[67] The Alberta Stroke Program Early CT Score, a 10-point score quantifying the signs of early infarction, also aims to improve patient selection.[68,69]

Therefore, in future trials, there should be a major effort to improve patient selection through the use of imaging criteria, in combination with other descriptors, (1) to select candidates who are expected to benefit from treatment, ie, those who have perfusion abnormality greater than diffusion abnormality, and (2) to exclude inappropriate patients, ie, those with lacunes or large infarcts with no perfusion-diffusion mismatch.[44,54,70,71] If imaging analysis cannot be performed online in the acute stage in time for decision making, the scans should be analyzed by prespecified criteria as soon as possible to select the target population; patients with no evidence of penumbra posthoc should then be excluded from an efficacy analysis.

Pitfall 3: Preclinical Studies Have Demonstrated Protection of Gray Matter, Whereas Clinical Trials Frequently Enroll Patients Without Specifying Location of Damage

A particular concern is that preclinical neuroprotective studies have concentrated almost exclusively on the protection of cerebral gray matter from ischemic injury; the effects of neuroprotective therapy on cerebral white matter tracts are largely unknown.[23,72] The human brain contains a greater proportion of white matter compared with the rat brain,[72] and the failure of some neuroprotective trials may be due to an inability of certain agents to protect against axonal damage.[23,32,72] Approximately one third of human strokes are small-vessel lacunes, yet adequate animal models of lacunar stroke are lacking.[32,72] Until such data are available, it may not be reasonable to expect lacunes or subcortical white matter infarcts to respond to neuroprotective therapy. The pathophysiology of ischemic injury in white matter is different than in gray matter, and treatment targets likely differ as well.[23,72,73] *N*-methyl-D-aspartate (NMDA) receptors, for example, are preferentially located at synapses rather than along axons.[74,75] Blockade of sodium channels, calcium, or alpha-amino-3-hydroxy-5-methyl-4-isoxasole-propionic acid (AMPA) receptors (rather than blockade of glutamate-mediated excitotoxicity) has been hypothesized to be more important for white-matter protection.[73,74] The NMDA antagonist MK-801 reduced cortical gray-matter injury but not the amount of axonal damage after middle cerebral artery occlusion in cats.[76] Interestingly, however, a recent study showed that axonal and myelin damage could be reduced in rats with the NMDA antagonist CNS 1102 (Cerestat).[74] More studies like this that take into account both gray-matter and white-matter pathophysiology are needed if we are to achieve "total brain protection."[72] For now, only cortically based strokes should be enrolled in neuroprotective trials, unless the agent being tested is specifically designed to protect white matter also.[16] Diffusion-weighted MRI is beginning to be used in some trials to select patients with cortical involvement and exclude those with lacunar infarction.

Pitfall 4: Optimal Duration of Neuroprotectant Administration Is Unknown

In recent trials, drug administration has varied from a single injection to continuous infusions to multiple doses lasting up to 3 months after stroke.[77] Because acute treatment may only delay but not prevent cell death, Dyker and Lees[77] advocate continuing neuroprotective therapy for at least the first 72 hours, if not longer. Prolonged elevation of excitatory amino acids after stroke in some patients[78,79] and MR spectroscopy evidence suggesting ongoing neuronal loss over many days after stroke[80] support the concept of extended treatment. In rats, longer-lasting neuroprotection was achieved when a

glutamate antagonist was given for 1 week compared with administration in only the acute phase.[31]

However, certain drugs may exert different or even opposite actions, depending on the timing of administration. NMDA antagonists, benzodiazepines, or barbiturates may be beneficial if given early after ischemia but may have detrimental effects if given at later times.[81–84] For example, GABA-ergic agonists may be neuroprotective when given acutely (a trial of diazepam within the first 3 days after stroke is currently underway[85]), yet may impair recovery if administered at later stages after stroke.[86] Additionally, neurite outgrowth requires both NMDA and voltage-sensitive calcium channel activation.[87] Because neurite outgrowth is likely an essential process during recovery, patients maintained on NMDA antagonists and voltage-sensitive calcium channel blockers may suffer impaired recovery. The time point at which the therapeutic transition from neuroprotection to repair occurs merits further study.

Pitfall 5: Preclinical Studies Have Relied on Infarct Size to Judge Therapeutic Efficacy, Whereas Clinical Trials Rely on Behavioral Outcomes

Traditionally, animal studies have relied on reduction in infarct size within the first few hours after stroke as the primary measure of therapeutic efficacy. In contrast, clinical trials judge efficacy by using neurological and/or functional outcomes, not infarct volume, most often at 3 months after stroke.[3,34] As an example, preclinical studies of the antineutrophil adhesion agent Hu23F2G (LeukArrest) and the glycine antagonist GV150526 (gabestinel; Glycine Antagonist in Neuroprotection study)[88] assessed infarct volume within hours of occlusion, while patients were evaluated on day 28 or 90, respectively, for neurological and functional outcomes. Such discrepancies suggest that reliance on infarct size measurement alone in animals can be misleading as an indicator of therapeutic efficacy.[89,90] Histological end points cannot tell whether surviving neurons are functional or dysfunctional or will go on to die in a delayed fashion, and they are less predictive of long-term histology than early behavioral assessments.[90] Moreover, some compounds [eg, basic fibroblast growth factor (bFGF), osteogenic protein-1] have been associated with functional improvement without affecting infarct size in animals, suggesting that they act by other mechanisms, eg, enhancement of neural repair, rather than by neuroprotection.[91,92]

Therefore, assessment of therapeutic efficacy in preclinical studies should require, in addition to infarct size, demonstration of benefit on functional measures of motor, sensory, or cognitive deficits.[89,93] Examples include tests of limb placing, beam walking, grid walking, Rotorod performance, grip strength, balance beam inclined plane performance, prehensile traction, and cognition (eg, Morris water maze, radial maze, 1-trial passive avoidance, T-maze retention test).[89,90,94] These measurements are not equivalent in their reliability or predictive value. For this reason, it is better to use a battery of appropriate tests rather than a single measure. The staircase test has been recommended because of its greater sensitivity in detecting persisting deficits in forepaw dexterity months after ischemia, unlike simpler sensorimotor tasks on which animals can recover quickly.[89,90] The bilateral sticky tape test may be a useful indicator of poststroke neglect.[94] Further development, refinement, and standardization of reliable functional assessments will continue to be a priority.[89,90,94]

Pitfall 6: Preclinical Studies Have Relied on Early Outcomes, Whereas Clinical Trials Rely on Late Assessments

Preclinical studies of neuroprotective drugs have rarely shown that early therapeutic benefit, when it is achieved, has a lasting impact. That is, of studies that have explored the long-term results when a drug showed early favorable influence on histological outcome, most have concluded that the early reduction in infarct volume does not persist if one continues to observe the animal; ie, most therapeutic attempts delay but do not arrest cell death.[26,90,95] For example, the NMDA antagonist MK-801 appeared neuroprotective at day 3, but at 4 weeks, there was no significant difference in infarct size.[95] Similarly, the cyclin-dependant kinase inhibitor flavopiridol, AMPA antagonist NBQX, and N-type calcium channel antagonist SNX-111 all appeared neuroprotective histologically when assessed at 1 week, but this was not sustained at 4 weeks.[96,97]

Such findings demonstrate that reliance on early end points is not sufficient and can be misleading; assessments at extended time points after ischemia are necessary to determine whether there is evidence of sustained neuroprotection.[15,26,95] Indeed, histopathological studies in animals show that infarcts evolve over time and may take many days to months to acquire their final appearance.[95,98,99] Late consequences of ischemia (eg, inflammation) or slow death mechanisms that are unleashed (eg, apoptosis) may in part explain such findings. Thus, if single-dose neuroprotective treatment only postpones the evolution of an infarct, perhaps multidose, extended treatments or combination therapy will be required for optimal neuroprotection (see below).[77,100]

Pitfall 7: Experimental Stroke Models Are Homogeneous, Whereas Human Stroke Is Heterogeneous

Another problem confounding the evaluation of neuroprotective therapy is the tremendous variability of human stroke types, recovery patterns, and associated clinical factors. Preclinical studies usually involve middle cerebral artery occlusion in young, healthy animals under anesthesia with tightly controlled temperature, blood pressure, oxygenation, and glucose levels ("interventional homeostasis"[54]).[101] In contrast, clinical trials often permit entry of multiple stroke types (cortical, mixed cortical-subcortical, pure subcortical white-matter strokes, and in some neuroprotective trials, both ischemic and hemorrhagic stroke), and there is a lack of standardized control over physiological parameters.[54] Unlike the animal model, stroke patients typically have a multitude of associated variables that may affect prognosis,[102–104] including old age, comorbidities, polypharmacy, recurrent ischemia, poor collateral circulation, or prior strokes. Hyperglycemia and other metabolic prognostic markers[105] may be particularly important variables to control or adjust for in future trials.[106–108]

TABLE 1. Influence of Outcome Measures on the Perception of Recovery*

Outcome Measure	Percent of Patients Considered Recovered,† %
Rankin 0–2	54
Rankin 0 or 1	24
Barthel >90	57
Barthel ≥75	40–50‡
Barthel ≥60	49–70‡
NIHSS 0 or 1	45
Fugl-Meyer >90	37
SF-36–PFI >75	28

SF-36–PFI indicates Short Form 36, physical functioning index.
*Data from the Kansas City Stroke Study (n=459), Duncan et al.[113]
†At 6 months after stroke.
‡From Duncan et al.[34]

Instead of viewing stroke as a single disease entity, future trials should more appropriately be directed only to specific homogeneous stroke subtypes. Currently, of 178 published acute stroke trials, only 2% specified a target stroke mechanism, and 35% specified a specific stroke territory.[3] Although a lack of specificity regarding stroke types in trial entry criteria may be appropriate for thrombolysis (where the pathophysiologic target, ie, clot, is similar regardless of stroke location, perhaps with the exception of lacunes), this may not be appropriate in neuroprotective trials in which efficacy in some patients (eg, cortical stroke), if present, could be diluted by the inclusion of other stroke types (eg, subcortical strokes). Indeed, posthoc analysis of some neuroprotective trials has suggested that a benefit may exist for certain subgroups, eg, patients with large cortical stroke [total anterior circulation stroke in the Clomethiazole Acute Stroke Study (CLASS)].[109,110] A follow-up study specifically targeting these patients failed to validate this hypothesis, however, but it set a good example for how we should approach future studies.[111]

Pitfall 8: Choice of Outcome Measures Can Determine the Success of a Clinical Trial More Than Actual Drug Efficacy

The choice of outcome measures in clinical trials is critical to the success or failure of a putative therapeutic intervention.[16,34,54] However, there is a lack of agreement about the most appropriate measures that should be used and about what constitutes "recovery" or "favorable outcome."[34,112–114] For example, on the Barthel Index, cutoff points anywhere between 50 to 95 of 100 have been used to define recovery.[115] Kidwell et al[3] showed that less than half of published acute stroke trials used a validated outcome measure and that only 17% had a prespecified primary end point. In the review of 51 phase II and III acute stroke trials by Duncan et al,[34] there were 14 different impairment level measures, 11 different activity (disability) measures, 8 miscellaneous scales, and only 1 quality-of-life measure. If disability scales are used (eg, Barthel Index, modified Rankin Scale), more patients will be considered recovered; if impairment scales are used (eg, NIHSS) fewer patients will be considered recovered.[34] Table 1 shows how one's impression of recovery is directly dependent on the type of outcome measure chosen.

This variability in outcome assessment has made the stroke literature appear confusing and at times conflicting. Indeed, much of the controversy regarding the thrombolytic trials has resulted from inconsistency in the definition of recovery and differences in end points used among the various trials. In the NINDS study,[4] the benefit of tPA at 24 hours did not reach statistical significance on the prespecified NIHSS end point. However, posthoc analysis showed that if recovery is instead defined as an NIHSS score of 0 to 2, a striking difference is found: 24% of tPA-treated patients versus 5% of placebo-treated patients are recovered at 24 hours.[116] Moreover, the 24-hour outcome results would have been statistically significant on the predefined primary end point if the recently published modified version of the NIHSS had been used instead.[117] Furthermore, in the European Cooperative Acute Stroke Study (ECASS II), which used "favorable outcome" as defined in the NINDS study (modified Rankin scale score of 0 or 1), the result was statistically negative.[118] However, when a different dichotomization that classifies outcome in terms of self-care independence (Rankin score of 0 to 2) was used, the study was positive.[118]

Thus, greater consensus and standardization in outcome measures for acute stroke studies are needed, and this would facilitate meta-analysis. It is recommended that future efficacy trials incorporate outcome assessments that span the spectrum of stroke recovery, ie, impairment, activity limitations (disability), and participation restrictions (handicap).[34] As suggested by Duncan et al,[34] a single scale likely is inadequate to capture recovery, and dichotomized outcomes should be avoided; inclusion of extended/instrumental activities of daily living assessments, advanced mobility measures, and quality-of-life assessments is recommended.[34] Although it may be appropriate in 3-hour thrombolysis trials to aim for neurological cures (eg, NIHSS score 0 or 1) or functional recovery (eg, modified Rankin score of 0 or 1), in trials of neuroprotective agents or longer time windows, our expectations should be different. Grotta[40] reminds us that we should be aiming for neuronal protection, not neuronal "reincarnation." Therapeutic efficacy will likely be of a smaller magnitude, one that may be captured only by using less stringent criteria for recovery (eg, NIHSS <7 or modified Rankin score of 0 to 2), or by measuring shifts in disability states.[118a] A newer end point, neurological deterioration in hospital, has been proposed for traumatic brain injury trials.[119]

Different measures of recovery may be necessary, depending on the severity of the stroke population under investigation (ie, mild, moderate, or severe), the particular type of treatment being studied, or the specific neurological function targeted by the intervention. For example, in a recent trial of stem cell implantation for hemiparetic stroke patients,[120] the global neurologic deficit scales used (NIHSS and European Stroke Scale) would not be expected to capture meaningful change in motor impairment; instead, a motor-specific impairment scale such as the Fugl-Meyer Stroke Assessment[121,122] might be more revealing given the location of the target stroke in the subcortical basal ganglia region. As we learn the limitations of existing scales, newer stroke-specific

indexes incorporating quality-of-life measurements are being developed; eg, the Stroke Impact Scale[123] and SS-QOL[124] are intended to provide more comprehensive and more meaningful outcomes from the patient's perspective.[124] Inclusion of specific scales for aphasia, neglect, or apraxia may reveal benefits in subgroups of patients that are not apparent on global deficit rating scales. The concept of separate "motor-Rankin" and "cognitive-Rankin" scales has been advocated.[125] Stratification of patients in clinical trials by initial stroke severity is important. The Orpington prognostic scale, recently shown to have excellent predictive value for stroke recovery, may have value for stratifying patients in treatment trials.[126] The optimal time for outcome assessment is debatable, 3 months after stroke (or earlier) according to some[16] and 6 months according to others.[34] In some studies, even if a therapeutic effect on final outcome (ie, at 3 or 6 months) cannot be demonstrated, it may be desirable to detect whether the intervention was able to accelerate the rate of recovery. Comparison of change scores (the difference between baseline and final scores for each subject) may have the advantage of minimizing interindividual variability in stroke severity and recovery if reliable outcome measures are used.

Pitfall 9: Small Trials Are Trying to Answer Questions That Only Large Trials Can Answer

Have some stroke trials been negative because of a lack of efficacy or because of a lack of statistical power? To detect efficacy of neuroprotective compounds, which are likely to have small rather than large treatment effects, we need large trials (thousands of patients, according to some experts) to prevent type 2 statistical error.[54,127] The mean sample size of neuroprotective trials has been 186 (median, 69).[3] Only 2% of acute stroke efficacy trials have had sufficient statistical power to demonstrate a 5% absolute clinical benefit, and only 7% of trials have been powered to detect a 10% benefit.[3] The use of "adaptive randomization" in future trials may reduce sample size requirements.[128] This Bayesian statistical technique, being used in a neuroprotective trial currently underway,[129] aims to maximize the number of patients assigned to the dose(s) that appear most efficacious; outcome data from each patient provides feedback to the randomization computer as the trial proceeds to optimize the chance that the correct drug dose will be studied.[54]

Part 2: New Therapeutic Frontiers

Most trials to date have attempted to modulate the early metabolic events in ischemia, particularly those involving glutamate activation of the calcium cascade. With a growing understanding of the pathophysiology of ischemic brain injury in the acute phase of stroke, as well as progress in understanding the mechanisms that underlie functional recovery in the subacute stages, newer therapeutic strategies are emerging.[81,130–134] The concept of a single narrow time window for intervention is being replaced by the potential for multiple overlapping therapeutic windows and the possibility of multiagent chemotherapy "cocktails" administered at selected time periods after stroke (Table 2).[130] In this section, we discuss a few selected examples of strategies that may form the basis for future trials, either alone or in combination with traditional neuroprotective approaches.

TABLE 2. Possible Stroke Treatment Options for the Future: Evolving Time Windows and Combination Therapy Approaches

Prestroke
Prophylactic neuroprotection for high-risk patients?
Minutes to hours after stroke
Acute reperfusion therapies
Intravenous thrombolysis
Intra-arterial thrombolysis
Combined intravenous/intra-arterial thrombolysis
Mechanical reperfusion techniques
Minutes, hours, or days after stroke
Neuroprotective therapy: chemotherapy cocktail of agents targeting different aspects of the ischemic cascade, perhaps administered sequentially at various time points after stroke
Antinecrotic agents
Antiadhesion/anti-inflammatory agents
Antiapoptotic agents
Combined thrombolysis and neuroprotective therapy
Tight control of glucose, perhaps insulin administration
Tight control of temperature, perhaps antipyretic administration, perhaps hypothermia
Days, weeks, or months after stroke
Restorative treatments targeting specific deficits, eg, gait retraining, arm function
Pharmacotherapy coupled closely with rehabilitation (rehabilitation pharmacology)?
Growth factors coupled with rehabilitation?
Stem cell therapy coupled with rehabilitation?
Gene therapy?
Ongoing
Avoidance of "detrimental" drugs
Secondary stroke prevention therapies, including combination antithrombotic agents

Delayed Neuronal Death After Ischemia: A Role for Antiapoptotic Therapy?

Observations of delayed neuronal death after ischemia have suggested the possibility of an "apoptosis-necrosis" continuum. Depending on the degree and duration of ischemia, brain cells may die by an ionic cascade, rapidly (necrotic cell death), or by a molecular cascade, slowly (apoptotic cell death).[131,132,135–137] At one end of the spectrum, severe focal ischemia produces infarction through excitotoxic necrosis usually evident in rats by 6 hours and maximal at 24 hours. At the other end of the spectrum, when ischemia is mild and short lasting, the resulting cell death may be apoptotic; ie, neurons appear to be spared initially but later go on to die slowly.[138] For example, after transient focal ischemia in rats Du et al[138] found no infarction at 24 hours; however, by 3 days, a small infarct had developed, and remarkably by 14 days, the infarct had progressed to the same volume as that induced by severe ischemia. Although excitotoxic necrosis is considered the predominant mechanism of ischemic cell

death in most cases, apoptosis may occur in penumbral neurons that escape excitotoxic death.[132] Importantly, if necrosis is attenuated by therapy (ie, by reperfusion or antiexcitotoxic agents), then apoptosis may be unmasked or even promoted.[132,136,138,139]

For these reasons, it is short-sighted to continue only with antinecrosis therapies without taking into account the role that apoptosis may play in ischemic cell death. Antiapoptotic agents (eg, cycloheximide, caspase inhibitors) have shown neuroprotective effects in animals in terms of both infarct volume reduction and functional improvement. Furthermore, the neuroprotection appears long lasting.[140,141] The therapeutic window for antiapoptotic neuroprotection is longer than that for most other neuroprotective agents (eg, 9 hours for the caspase inhibitor ZVAD-fmk and zDEVD-fmk after brief ischemia and up to 12 hours if combined with an NMDA antagonist).[142] The role of apoptosis in human stroke, however, and the clinical relevance of antiapoptotic therapy are not yet known and await further investigation. If further studies confirm the occurrence of apoptosis in the penumbra region, then cerebral blood flow measurement may become a clinically accessible surrogate marker for this outcome.

The Need for Polytherapy

On the basis of the complexity of events in cerebral ischemia and the disappointing results from single-agent trials, it may not be realistic to expect that 1 neuroprotective drug will have lasting benefit. Rather, effective neuroprotection may require "rational" polytherapy that combines drugs with different mechanisms of action, perhaps administered at different poststroke intervals, to maximize efficacy and/or extend the window for reperfusion, minimize reperfusion injury or hemorrhage, or inhibit delayed cell death.[16,130,143,144] Furthermore, because the failure of several neuroprotective trials has been attributed to dose-limiting toxicity,[145] combination therapy may permit lower doses of each agent and minimize adverse effects. Combinations with nonpharmacological (physiological) neuroprotective strategies such as hypothermia, insulin, and blood pressure control should also be subjected to clinical trials.[103,146]

Combined thrombolysis-neuroprotective approaches have shown promise in animal studies and are beginning to be investigated in clinical trials. For example, synergistic effects have been demonstrated in animals when thrombolysis is combined with citicoline,[147] an AMPA antagonist,[148] an NMDA antagonist,[149] or other agents.[150,151] Administration of antileukocytic adhesion antibodies has been shown to extend the therapeutic window for thrombolysis.[152,153] Recently, 2 trials have demonstrated the feasibility and safety of intravenous tPA treatment followed by neuroprotectant administration: the CLASS-T trial of clomethiazole[154] and a study of lubeluzole.[155]

In animals, synergy has been demonstrated by the combination of 2 neuroprotective agents with different actions; some examples include the NMDA antagonist MK-801 in combination with a GABA agonist,[156–158] a free radical scavenger,[159] a calcium antagonist,[160] citicoline,[161] or bFGF.[162] Similarly, synergy has been observed with the combination of the antioxidant tirilazad and magnesium,[163,164] the combination of 2 different antioxidants,[165] and citicoline combined with bFGF.[166]

Synergistic effects on infarct size and therapeutic window have also been found in animals through the use of antiexcitotoxic agents in combination with antiapoptotic agents, eg, the NMDA antagonist dextrorphan plus the protein synthesis inhibitor cyclohexamide,[167] or MK 801 with the caspase inhibitor ZVAD-fmk.[168] Caspase inhibitors given with bFGF extended the therapeutic window and lowered the required doses for neuroprotection.[169]

Beyond Neuroprotection: Exploiting the Repair Mechanisms of the Brain

The poststroke recovery period (days, weeks, and months after stroke) represents another target for more active therapeutic development. Our preoccupation with hyperacute and acute intervention, while critically important, should not lead us to neglect the testing of "restorative" interventions that might enhance recovery in the subacute and chronic stages after stroke.[81,170] Unlike neuroprotective therapy that targets ischemic tissue and aims to limit infarct size, "recovery-enhancing drugs" and other novel interventions for stroke aim to promote functional recovery after a completed stroke by stimulating repair mechanisms.

The Changing Milieu of the Brain After Stroke

Within minutes of ischemia, a loss of dendritic spines can be observed at excitatory synapses; reestablishment of dendritic spine synapses in surviving neurons can occur rapidly and represents a potential substrate for functional recovery.[171] Ischemic cortical injury induces the expression of growth factors in peri-infarct regions, and behavioral recovery is accompanied by increased dendritic branching and synaptogenesis that peaks 2 to 4 weeks after stroke in the rat.[172,173] Mechanisms of neuroplasticity postulated to underlie recovery include unmasking of latent connections, redundancy that allows recruitment of alternate parallel pathways to take over lost functions, axonal sprouting from surviving neurons with formation of new synapses, and possibly even neurogenesis.[134,174–176a]

Intracisternal administration of bFGF beginning 24 hours after stroke or osteogenic protein-1 beginning 3 days after stroke promoted recovery in animals without affecting infarct size and stimulated new neuronal sprouting and synapse formation.[91,92,177,178] Clinical trials of bFGF administered within 6 hours have been underway, but 1 trial was terminated early because of safety concerns.[179,180]

Proteins that are normally expressed in the developing brain and suppressed in the adult brain become upregulated in response to ischemia; stroke "recapitulates ontogeny," as summarized by Cramer and Chopp.[181] Cell-cycle genes are re-expressed in response to ischemia; ie, neurons are activated to "divide," but they are hard wired not to. Activation of the cell cycle cascade results in cell death, and interrupting this cascade results in cell survival.[182] This may be another fruitful therapeutic avenue in ischemia. Stem cell therapy is being investigated for enhancement of stroke recovery. There are suggestions in the literature that bone marrow stromal cells or umbilical cord blood cells injected intravenously or

intra-arterially in rats can migrate toward the infarct, stimulate growth factors, and promote functional recovery.[183–185] Recently, human experimentation has begun with a phase I trial of transplantation of cultured neuronal cells into basal ganglia infarcts in stroke patients.[120] A great deal more animal work is needed to provide a firmer basis for intervention.

Remapping and Rehabilitation

Cortical reorganization after stroke is promoted by rehabilitation and an enriched environment.[134,186,187] Nudo et al[188,189] showed that in monkeys who do not receive rehabilitative training after a small focal injury in the hand motor cortical region, the surrounding intact cortical representation undergoes shrinkage; however, with repetitive training in the form of motor skill acquisition, behavioral recovery is promoted, and the cortical hand representation is maintained or expanded. Thus, physical therapy may derive its effectiveness from "teaching" the brain to learn. Improved recovery in humans may be achieved with increased intensity of rehabilitation (more hours and greater frequency of therapy).[190] Such dose-response effects suggest the concept that "more is better" applies to stroke recovery. One intensive physical therapy regimen that is gaining popularity is "forced use" or constraint-induced movement therapy, which can be applied if the weak arm has enough strength to at least move against gravity.[191] In this protocol, the unaffected arm is constrained in a sling, forcing the patient to use the affected arm as much as possible in meaningful daily activities. The aim is to minimize the development of learned helplessness resulting from overreliance on the "good arm." Based on reported success in chronic stroke patients, this therapy is now under investigation in multicenter trials as a rehabilitation intervention in the earlier stages after stroke. Bilateral arm use assisted by passive movement may enhance activation in the peri-infarct region of a stroke[192] and may have a role in early rehabilitation. Body weight–supported treadmill training is a promising technique being investigated to promote recovery of ambulation after stroke.[193]

Pharmacological Manipulation After Stroke

Recovery after stroke may also be modulated pharmacologically.[81,178,194–196] Accumulating evidence suggests that the recovery process is dynamic and vulnerable to neurotransmitter modulation.[82] For example, pharmacological studies in animals have emphasized the importance of central noradrenergic transmission in mediating some forms of recovery after focal cortical injury. Drugs augmenting noradrenergic activity (eg, dextroamphetamine) enhance functional recovery when coupled with symptom-relevant experience, whereas drugs decreasing noradrenergic activity impair recovery and can reinstate deficits after recovery has taken place.[197–200] (For a review of additional studies, see Reference 170.) Similar findings have been demonstrated for other classes of drugs and may relate to their ability to facilitate (or impair) long-term potentiation.[82,201] Histologically, dextroamphetamine administration after cortical infarction in rats has been associated with upregulation of neural sprouting and synaptogenesis in the peri-infarct cortex and contralesional cortex, correlating with behavioral recovery.[202,203] In animal models, chronic amphetamine administration, coupled with another stimulus, has been shown to increase cortical responsiveness possibly through upregulation of CREB, a protein transcription factor, and induction of genes mediating other molecular changes.[204] Experiments from Leonardo Cohen's laboratory investigating the effects of various drugs on cortical plasticity have shown that dextroamphetamine administration facilitates the induction, magnitude, and retention of use-dependent plasticity in humans during performance of a motor training task.[205]

The concept of rehabilitation pharmacology, which dates back >20 years,[206] proposes that conventional physical, occupational, or speech/language therapy might be augmented if coupled with pharmacotherapy to enhance activity-dependent plasticity.[207] On the basis of small clinical studies showing some promise,[208–210] we are conducting a clinical trial in Canada to investigate the effects on motor recovery of dextroamphetamine versus placebo coupled with physiotherapy after hemiparetic stroke,[211] and in the United States, a multicenter trial of amphetamine-facilitated recovery is underway. A recently published study, however, showed no benefit of amphetamine on motor recovery in a randomized, controlled trial of 36 patients treated with intermittent doses of drug coupled with physical therapy beginning 5 to 10 days after stroke.[212] However, racemic amphetamine 10 mg was used instead of dextroamphetamine as in previous studies, and their patients were older than in previous studies (average age, 80 years). Stroke type and neuroimaging characteristics that might influence recovery or treatment response (eg, lesion size, location) were not reported, and patients with moderate hemiparesis were not analyzed separately in comparison to those with severe hemiparesis. The effects of dextroamphetamine coupled with speech/language therapy are also being investigated in ongoing studies by Walker-Batson et al[213–215] and have shown some promise for enhancing aphasia recovery. Trials of other noradrenergic agonists (methylphenidate, L-DOPS), levodopa, and fluoxetine have been conducted recently and provide further proof of concept for the strategy of poststroke rehabilitation pharmacotherapy.[216–219]

Goldstein et al[195,220,221] have drawn attention to the observation that several drugs shown to be detrimental in the laboratory are commonly prescribed to hospitalized patients after stroke and head trauma and may have similar detrimental effects on recovery in humans. These include the antihypertensives clonidine and prazosin (α-noradrenergic antagonists), haloperidol and other dopamine antagonists, benzodiazepines, phenytoin, and phenobarbital.[194,195] Retrospective reports suggest that exposure to such drugs is associated with poorer motor recovery, independent of the severity of initial deficit or comorbid conditions.[222,223] Aphasia recovery may also be impaired by certain drugs.[206,224] In a prospective study of chronic stroke patients, administration of increasing doses of a benzodiazepine reinstated previously recovered focal deficits (hemiparesis, aphasia, neglect).[86]

Clinical rehabilitation trials are promising for many reasons and may avoid some of the methodological obstacles encountered in many acute stroke trials.[125] (Table 3) Preclinical studies of restorative therapies necessarily rely more on behavioral outcomes and extended follow-up periods (weeks

TABLE 3. Some Potential Advantages of Clinical Trials of Restorative Therapies*

More homogeneous patient population
Tighter inclusion criteria (more accurate diagnosis of stroke type before entry through imaging criteria)
Reduced variability because patients with early spontaneous improvement can be excluded (to concentrate on those patients with persisting deficits in need of rehabilitation and avoid a placebo responder effect)
Extended time windows
Less pressure of time to enroll patients
More detailed baseline and outcome assessments can be carried out
Wider applicability than acute intervention
More patients are eligible for rehabilitation interventions

*Modified from Finklestein.[125]

after stroke), making these animal models potentially more relevant to the human condition than models of acute neuroprotective therapy. Without the constraints of a narrow time window, effective restorative therapies may be able to reach many more patients than would qualify for acute treatment. The challenge remains, however, to design these trials well, select appropriate outcome measures, determine the clinically important difference, and power the samples accordingly.

Conclusions

Researchers and clinicians must become more cognizant of the pitfalls and paradoxes that have arisen in attempting to translate the results of animal studies into clinical trials of neuroprotective stroke therapy. Much needed recommendations to improve the quality of preclinical and clinical drug development have been published recently by the Stroke Therapy Academic Industry Roundtable (STAIR)[15,16] and others[20] and should be followed. Preclinical evaluation of therapeutic efficacy based solely on measuring infarct volume in the early phase is no longer adequate. Clinical trials should be based on preclinical evidence demonstrating improved functional outcomes at long-term end points measured on standardized batteries of validated behavioral tests. Assessment of neuroprotection should rely more on delayed time windows for drug administration, longer durations of ischemia, and models that take into account the protection of cerebral white matter in addition to gray matter.[15,16,72] Ideally, there should be converging evidence from >1 laboratory, different stroke models, and >1 animal species.[15]

Previous neuroprotective trials have not been selective enough in targeting homogeneous patients with cortical stroke and evidence for the presence of ischemic penumbra. Future trials need to use stricter entry criteria to target not just cortical strokes but specifically those with a sufficient volume of penumbra as determined by imaging. Although functional imaging is currently restricted to specialized centers, we urgently need proof of principle that neuroprotectives can work in humans if administered to the appropriate patient populations. Perfusion imaging with CT is worthy of further investigation because of the availability and practicality of CT for acute stroke imaging. With improved methods of patient selection, it is anticipated that future treatments will more appropriately be customized according to an individual's "penumbra window" rather than a rigid time window. Patient selection should be "penumbra-specific" for acute treatment interventions and "deficit-specific" for restorative/rehabilitative interventions. Greater consensus on outcome measures is needed because the choice of outcome measure remains highly variable and can determine the success or failure of a putative therapeutic agent.

If current neuroprotective approaches only delay rather than arrest cell death, future strategies may require "rational" polytherapy that combines drugs with different mechanisms of action, perhaps administered at different poststroke intervals and in combination with reperfusion strategies. The concept of the therapeutic window is evolving from a single rigid and narrow time period to multiple potential overlapping and sequential windows, spanning minutes, hours, days, weeks, and months after stroke. Although most trials have concentrated on excitotoxicity, newer targets, including inhibition of inflammatory reactions and apoptosis, are being explored. The time point at which the therapeutic transition from neuroprotection to repair occurs merits further study.

Stroke is a chronic condition, and we must not ignore opportunities for therapeutic intervention in the subacute and chronic stages. With a growing understanding of the mechanisms that underlie recovery, research directed at enhancement of neural repair and rehabilitation should be a high priority.

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David J. Gladstone, Sandra E. Black and Antoine M. Hakim
for the Heart and Stroke Foundation of Ontario Centre of Excellence in Stroke Recovery

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