Pharmacologic Interventions for Stroke
Looking Beyond the Thrombolysis Time Window Into the Penumbra With Biomarkers, Not a Stopwatch

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Background and Purpose—The majority of pharmacological agents for stroke were developed based on the assumption that neurological deficits will be reduced upon the successful interruption of biochemical mechanisms leading to neuronal death. Despite significant evidence of preclinical efficacy, none of these agents succeeded. They either failed to demonstrate efficacy in the clinic or their development was halted for safety, strategic, or commercial reasons.

Summary of Review—This “neuroprotection strategy” has focused primarily on targets in the neurotoxic environment that occurs under ischemic conditions. In many cases, these agents were designed to tackle events that are known to start almost immediately after onset of ischemia, which is far before a realistic therapeutic time window opens for most, if not all, patients with stroke. In other instances, they were evaluated beyond a realistic timeframe in which one could expect significant salvageable tissue or penumbra to exist. Surprisingly, most of these agents were not evaluated in conjunction with strategies for improving perfusion to the affected tissue, indicating an overoptimistic assumption that neuroprotection alone could be sufficient to halt injury caused by an abrupt interruption of brain blood flow.

Conclusions—We provide a constructive translational medicine perspective about how one could improve the drug development process with the hope that the probability for success can increase in our quest to establish a novel therapy for stroke. (Stroke. 2009;40:e558-e563.)

Key Words: biomarkers ■ drug development ■ ischemia ■ neuroprotection ■ translational medicine

There is a striking disparity between the large number of neuroprotectants that significantly reduce infarction in rodent models of ischemic stroke and their uniform failure in large clinical trials conducted thus far. Thus, it is not surprising that many are filled with negativity toward the prospects of producing the long-awaited therapy that could reduce the sequelae of ischemic brain injury. Although undue pessimism is not warranted, a reappraisal of our strategic approaches certainly is necessary. In this regard, The Stroke Therapy Academic Industry Round Table (STAIR) gathered recommendations from many key scientific and clinical leaders in the field. Although these recommendations did not provide a written guarantee for success, STAIR has identified important, logical issues that warrant careful consideration by academic and pharmaceutical investigators engaged in drug discovery and preclinical or clinical stroke research. These recommendations were expected to define an optimized approach that would produce an efficacious neuroprotectant for stroke. Unfortunately, after 5 sessions of continually updated STAIR criteria, no drugs have successfully made it through clinical trials and into practice.

There has been much debate about the reasons that could help explain this lack of success. These range from a flawed rationale for target selection, to pharmacokinetic and brain penetration issues, to design of preclinical studies and clinical trials, to timing of agent administration, to the predictive power of animal models. In general, the quality of published preclinical work has improved significantly over the past decade, much to the credit of STAIR. However, the discordance between animal studies and clinical trials of neuroprotective agents persists. This suggests that further improvements, if not major changes, are needed in experimental studies that support initiation of large clinical trials. In many cases, the rationale for transitioning drug candidates from the discovery stage into clinical development should have been questioned because preclinical experimental data were often not sufficiently robust to warrant testing in humans. For instance, many clinical trials involved interventions beyond 6 hours, a period that is not congruent with the timing of specific neurochemical events that were supposed to be halted by neuroprotective interventions based on their proposed mechanisms of action. In addition, imaging-based information about the presence of potentially salvageable ischemic brain tissue in enrolled patients was absent, which even further eroded the probability of success.

At the most recent STAIR meeting (March 2008), there was much discussion of ways to continue improving preclin-
ical studies to make them more congruent with clinical trials. These discussions focused on the need for randomization, the blinding of dose treatments and data analysis, a consideration of the neurovascular unit as the ultimate target for intervention, and the use of additional models, particularly nonhuman primates. We continue this discussion by examining additional methodological problems as follows.

How Should Development of Neuroprotective Therapies for Stroke Be Pursued?

The concept of pharmacological neuroprotection has evolved over the last 30 years to describe attenuation of multiple pathophysiological mechanisms that lead to cell death and tissue infarction. Many of these were considered highly relevant for therapeutic intervention purposes. Several classes of compounds capable of blocking some of these pathways were discovered, tested in preclinical models, and subsequently evaluated in clinical studies with disappointing results. For instance, glutamate-mediated excitotoxicity was considered a central event in ischemic injury based on a large amount of experimental work. Several glutamate receptor antagonists were developed and tested but failed to provide clinical benefit. We now recognize that excitotoxicity likely contributes to ischemic cell death, but perhaps it is not the most appropriate target for intervention in human stroke. Three key considerations could help explain the weakness of this theory. First, ischemia triggers the release of not only glutamate, but also of other neurotransmitters causing their transient, short-lived extracellular accumulation. This happens very early after stroke, which is far before therapeutic intervention is possible. Second, the therapeutic importance of excitotoxicity was build based on the ability of NMDA/glutamate receptor inhibitors to protect primary cultured neurons exposed to oxygen/glucose deprivation. This paradigm mimics some aspects of ischemic stress, but primary neurons tend to resist long periods of low oxygen and glucose. Also, cultured neurons do not undergo key pathophysiological events such as anoxic depolarization that are characteristic of the ischemic brain. Third, the efficacy of NMDA/glutamate receptor antagonists reducing ischemia-induced tissue damage was evaluated mostly in rodent models and often following clinically unrealistic treatment paradigms. Again, many classes of compounds known to be effective in treating rodent brain ischemia were not efficacious in patients with stroke.

Certainly, delayed intervention times, often unavoidable in humans, contributed to these and other failures; but perhaps more important is the fact that the pathophysiological significance of processes such as excitotoxicity was not well established before large-scale and expensive clinical efforts were initiated. It is plausible that we have not properly identified and characterized yet the most clinically relevant mechanisms of cell death that operate in the first hours of stroke when salvageable tissue evolves into the infarction. For instance, an emerging player in ischemic brain injury is the membrane hemichannel pannexin-1 that carries ions and signaling molecules between the cytoplasm and the extracellular space and might contribute to peri-infarct depolarizations and delayed cell death. Its relevance in ischemia-mediated injury remains largely unexplored.

On the other hand, blocking a single deleterious event might not be sufficient to reduce the sequel of a multifactorial condition like ischemic stroke. The list of processes involved in cell death and tissue infarction is diverse. These cellular events likely follow variable time courses that may or may not overlap differentially with each other. In this scenario, specific individual treatments effective at certain times under a given range of physiological conditions can be ineffective at others. Thus, it is tempting to speculate that interventions targeting multiple pathways, administered early in the injury process and that display a sustained effect spanning significant time postinjury, will be more effective in the context of a complex neurological disease such as stroke. Nevertheless, for this alternative approach to be practical, proper characterization of relevant pathways will be essential.

Figure 1 illustrates known mechanisms that are thought to participate in ischemic brain injury. A thrombus or embolus occludes a cerebral artery leading to an abrupt reduction in perfusion to the territory affected. In the absence of collateral flow, perfusion decreases to a critical level in the core ischemic area. In this core region, oxygen tension drops markedly, mitochondrial electron transport ceases, oxidative phosphorylation is halted, and ionic regulation stops. Under these conditions of ischemic hypoperfusion, the levels of high-energy phosphates that drive membrane ionic efflux pumps fall within minutes, resulting in increased neuronal intracellular concentrations of sodium and calcium, which are constantly driven from the extracellular to intracellular compartment by their concentration gradients. In addition, extracellular potassium increases significantly. The resulting anoxic depolarization triggers a massive release of neurotransmitters from synapses, especially large amounts of glutamate at excitotoxic concentrations. These effects are further amplified by the opening of sodium/calcium channels that are coupled to the maximally stimulated glutamate receptors. These considerable ionic shifts result in cytotoxic edema and a relatively rapid loss of cellular integrity. In addition, the rising cytoplasmic concentration of calcium activates phospholipases, proteases, and endonucleases resulting in extensive breakdown of cellular phospholipids.
proteins, and nucleic acids. Moreover, free radicals are produced in the process and contribute to oxidation of membrane lipids, proteins, and nDNA further amplifying cellular demise and triggering cell death pathways (i.e., apoptosis and/or necrosis).

The timing of these interdependent events have been studied experimentally and shown to be influenced by many factors, including the degree and rate of change in oxygen tension, pH, temperature, and changes in energy depletion/consumption. Thus, “validation” of a neuroprotective agent in a carefully controlled animal model only demonstrates activity in a narrowly defined range of physiological conditions, a well-defined timeframe, and only against a single target. Clinical stroke obviously encompasses a much wider range of changes, variables, and between-subject differences than in the controlled animal laboratory setting. Time after occlusion is, therefore, only a rough surrogate for defining which pathophysiological mechanisms are at play in any given ischemic brain region. Timing, although useful as a rough rule of thumb for a busy clinical service, is a poor substitute for careful definition of a patient’s individual clinical state, particularly when treatment options are being considered. Furthermore, ischemic changes affect not only neurons, but many cell types, including glial cells, smooth muscle and endothelial cells, leukocytes, and platelets. Each of these cell types undergoes a range of different changes during ischemia. They also respond differently to signaling molecules/mediators and have different classes of targets that could be modulated. Finally, the “no-reflow” phenomenon due to microvascular occlusion by aggregated platelets and leukocytes is another complication that needs to be taken into consideration because it could compromise reperfusion even after resolution of the precipitating arterial occlusion. When one considers all these multiple mechanisms and targets, and their temporal heterogeneity and complexity, it is not surprising that so far, clinical studies of neuroprotective interventions for ischemic stroke appear futile. It is encouraging that the neurocentric view of the problem is losing ground with more emphasis being given to the importance of the neurovascular unit as a key target of future stroke therapies.

**Acute Stroke Intervention Can Only Save the Salvageable: Treat the Penumbra!**

The current dogma regarding the timing of acute events poststroke in the context of physiological conditions used for experimental studies is illustrated in Figure 2. Clearly in the ischemic core, the effects associated with many neuroprotection targets start immediately and progress rapidly probably in <1 hour after stroke onset. In this region, a rapid drop in high-energy phosphate levels causes failure of the Na+/K+ ATPase pump within minutes as indicated by recordings of anoxic depolarization in experimental models. Once depolarization occurs, ionic concentration gradients across the neuronal membrane remain compromised and the inability to repolarize triggers rapid cell death. Even if many effects are still ongoing at the time of patient availability for treatment, the damage associated with such mechanisms likely has already been done. However, in the surrounding area where perfusion is partially maintained by dilatation of patent vessels and blood supply from neighboring collateral arteries, the tissue is metabolically compromised, electrically hypoactive, or “silent,” but is still viable. This heterogeneous region survives orders of magnitude longer than the core ischemic areas and is referred to as the ischemic penumbra. The concept of the ischemic penumbra (named after the halo surrounding an eclipse) originated decades ago and is broadly defined as an area of potentially restorable, severely hypoperfused tissue around the ischemic core. Only the cells in the penumbra, served by low/misery residual perfusion, can be salvaged within a reasonable time after ischemic stroke.

In cerebral infarction, necrosis is the primary model of cell death in the infarct core, whereas apoptosis and necrosis can occur in the penumbral area. Both mechanisms are dependent on the generation of reactive oxygen species and perhaps other mechanisms that remain poorly understood. Again, an
important event that often goes unrecognized is the presence of peri-infarct depolarizations (also called cortical spreading depression) that repeatedly stress the surviving but starved penumbral tissue during several hours after stroke onset. These peri-infarct depolarizations essentially recapitulate the one-time anoxic depolarization that arises within the ischemic core, except that some penumbral tissue can still repolarize after these events. However, this recovery is transient and depolarizations further contribute to energy depletion diminishing the ability of viable cells to withstand the next wave of depolarization. Just as important, each depolarization wave is associated with an inverse hemodynamic response in affected neurovascular units causing local vasoconstriction rather than vasodilation. Understanding the neurochemical events underlying peri-infarct depolarizations can potentially guide the design of novel approaches to improve tissue perfusion and inhibit infarct expansion.

The acute destructive events described are variable and occur rapidly. Later events also exhibit increased diversity and complexity as genomic responses trigger a host of reactions and signaling pathways, including inflammation, wound healing, and brain repair/remodeling (Figure 2). In any event, the destruction in the ischemic core is typically complete and irreversible well before intervention poststroke is possible. The importance of early intervention in stroke (ie, the adage “time is brain”) has been and continues to be emphasized. The most important data supporting the relevance of salvageable brain tissue (penumbra) are the beneficial effect of early recanalization and reperfusion achieved with thrombolytic agents or mechanical clot-removal devices. Early intervention can restore perfusion of the ischemic territory and results in salvage of penumbral tissue, prevention of infarct growth, and subsequent reduction of neurological impairment. Undoubtedly, recanalization approaches are not always successful in restoring, flow but still, thrombolysis using tissue plasminogen activator remains the only approved intervention for ischemic stroke. We have certainly learned valuable lessons from their clinical use (Figure 3).12,17

Perfusion and Diffusion Imaging: Seeing Is Doing

Rigorous identification of the penumbra requires definition of regions of reduced cerebral blood flow that have an increased oxygen extraction fraction13 and their distinction from the ischemic core where oxygen extraction is greatly diminished or absent. Such demonstration requires positron emission tomography scanning, not readily available in most hospitals. An alternative approach to penumbral imaging, first operationally defined over a decade ago, involves comparison of diffusion-weighted (index of cytotoxic edema and eventual tissue infarction) and perfusion-weighted (index of tissue perfusion deficit) MRIs. Although diffusion-weighted imaging can measure the severely ischemic, soon to infarct or already dead tissue, perfusion-weighted imaging provides an approximation of the extent of blood flow impairment. Conceptually, the mismatch between these regions, that is, the region where there is a perfusion-weighted imaging deficit but not a diffusion-weighted imaging abnormality, delineates the penumbral region with low flow/misery perfusion. This approach can allow monitoring the evolution of brain injury over time and thus can identify the degree of salvageable (ie, penumbral) tissue at any time post-stroke.10,11,13 Some clinical studies have documented that as many as 80% of patients might display a mismatch in the first 6 hours of stroke onset, and possibly as many as 40% of patients could still have some degree of penumbral tissue at 24 hours.14 If confirmed, these observations raise the hope that neuroprotection might be a viable approach over a wider time window than previously appreciated.

MR-based multimodal imaging techniques are certainly not infallible. They require further refinement. In some cases, diffusion-weighted imaging lesions are reversible and perfusion-weighted imaging indices cannot distinguish disabling perfusion deficits from benign oligemia. The para-
ters used to define perfusion mismatch remain somewhat arbitrary, differ from center to center, and do require further standardization.\textsuperscript{14} Despite this variability, mismatch changes do represent the growth of infarcted tissue over time (ie, a transition from viable penumbral tissue to infarction) and this has been validated by other direct tissue measurements.\textsuperscript{13} Current data support mismatch as the best clinically available technique for the identification of potentially treatable patients.

An alternative technique that is gaining attention in stroke clinical centers is a modification of CT called perfusion CT, which is less expensive, faster, and more widely available than MRI. Perfusion CT delineates the ischemic tissue (penumbra) by showing increased mean transit time with decreased cerebral blood flow and normal or increased cerebral blood volume, whereas infarcted tissue manifests with markedly decreased cerebral blood flow and decreased cerebral blood volume. A modern CT examination also includes CT angiography that can depict the occlusion site, help grade collateral blood flow, and help characterize large artery atherosclerotic disease. A complete CT study (nonenhanced CT, perfusion CT, and CT angiography) may be performed and analyzed rapidly and easily by general radiologists using a simple standardized protocol. It may even facilitate diagnosis by less experienced radiologists in affected patients. Although perfusion CT still requires further refinement, current clinical data suggest good correlation with other estimates of blood flow deficit, extent of penumbra, and final infarct size.\textsuperscript{15}

The impact of imaging techniques is certainly not restricted to clinical applications. Preclinical studies that incorporate techniques such as MRI mismatch analysis have the potential to improve congruency of animal models with human stroke. In this regard, the use of imaging biomarkers could play a pivotal role in the discovery and development of drugs for ischemic stroke. For instance, establishing experimental models that display a pattern of penumbral evolution comparable to human salvageable tissue could serve as a key platform to determine drug efficacy. This simple effort could help increase significantly the confidence that a drug candidate might have potential as a therapeutic agent for human stroke.

How Should We Advance Neuroprotection for Stroke?

Neuroprotection will never be more than an adjunctive intervention to reperfusion of the penumbra that occurs either spontaneously or induced by thrombolysis. Nevertheless, properly applied, it would be a useful intervention as part of the overall therapeutic management of stroke. A limitation of “neuroprotection” as a therapeutic strategy is the assumption that lasting protection can be achieved even in the presence of continued hypoperfusion. Given the instability of the clinical condition, with multiple opportunities for exponential deterioration, such prolonged neuroprotection might not be a realistic expectation. Although neuroprotection can be expected to increase tissue resistance to the “misery perfusion,” to prolong cell survival and to delay activation of irreversible cell death pathways in the penumbra, the ischemic environment itself must be resolved and homeostasis must be restored. If not, the result eventually will still be massive cell death and infarct expansion (see Figure 3A versus 3B).

In clinical stroke, time is of the essence, but timing and choice of interventions ideally would be dictated not by a standard “one-size-fits-all” clock that ticks from the often

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**Figure 3.** Hypothetical scenarios for progression of ischemic brain injury after stroke. A, If no treatment is attempted, infarct evolves rapidly leading to permanent damage. B, If a neuroprotective drug is administered but the ischemic environment is not altered, the infarct maturation/growth will progress and eventually reach its maximum size/volume. A neuroprotective agent might slow the progression of infarct, but this salubrious effect will eventually be overcome by the persistent ischemia that maintains a vicious cycle of deleterious neurochemical events. C, The progression of infarction could only be reduced by interventions that either improve perfusion to the penumbra (eg, approaches that enhance collateral flow) or resolve the ischemic environment (eg, thrombolysis). In the case of neuroprotective agents, their sustained benefit will depend on reperfusion either spontaneous or therapeutically induced. Importantly, neuroprotective and/or vascular protective agents might be able to maintain sufficient tissue integrity that could allow an extension of the time window for thrombolysis.

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poorly estimated time of symptom onset, but from direct assessment of the compensatory and pathophysiological mechanisms at play when the patient with stroke presents for treatment. A useful first step in this direction has already been taken by the admittedly crude, but nevertheless useful, technique of diffusion-weighted imaging/perfusion-weighted imaging mismatch MRI, as has been discussed. Patients without penumbra can be spared the hazards of futile treatment and only those patients with salvageable penumbra enrolled in clinical trials. Can we do even better? More accurate distinction of ischemic core from penumbra has already been promised by blood oxygenation level-dependent MRI techniques, but not yet proven. Existing technologies permit measurement of phosphorylation potential and pH in living brain. However, these must be made more rapid and accessible if they are to be useful during the inescapable rush to treat ischemic stroke quickly. Furthermore, accessible biomarkers of vascular fragility and of released cytotoxins are either in their infancy or still need to be discovered.

The dynamic range, the capabilities and limitations, of novel neuroprotective agents must be defined more fully in experimental models if they are to be used intelligently in the clinic. In this regard, development of biomarkers will permit selection of patients suitable for specific treatments that target any of a range of potentially neuroprotective mechanisms. More importantly, such biomarkers, many as yet undiscovered, will not only identify suitable recipients, but also will guide dose selection and provide early readouts of efficacy. Undoubtedly, studies using such biomarkers will demonstrate that many of the mechanisms that we currently consider important turn out not to be. Others will emerge.

To implement such a mechanistic biomarker strategy, we must learn to be systematic, resolving one question at a time. Our ultimate goal is a treatment sufficiently robust to be available quickly to the millions of stroke victims who are in ambulances and emergency rooms. However, we are only likely to achieve that goal by systematic studies of highly selected and intensively monitored patients in specialist intensive care stroke centers. The steady incremental improvements in biomarkers developed over the decades by our colleagues in the oncology field attest to the value of perseverance and the systematic approach.

Disclosures

None.

References

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