New Approaches to Neuroprotective Drug Development

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Abstract—All prior drug development programs of neuroprotective agents were unsuccessful for a variety of reasons related to both preclinical assessment and the design/implementation of clinical trials. The neuroprotection hypothesis of improving functional outcome related to salvaging ischemic brain tissue is strongly supported by robust preclinical data for many agents. In the future, monotherapy neuroprotection trials will be difficult but could be performed in underused centers with drugs that have very promising and complete preclinical results. Additional approaches for the testing and use of neuroprotective agents should be considered. Novel approaches would include extending penumbral survival for the later use of reperfusion therapy, reducing reperfusion injury after successful reperfusion, and using drugs with both neuroprotective and recovery enhancing effects, as exemplified by granulocyte colony-stimulating factor and citicoline. To maximize outcome after stroke, the combined use or reperfusion and neuroprotection is likely to be needed, so we must begin to perform carefully designed trials with this combination. (Stroke. 2011;42[suppl 1]:S24-S27.)

Key Words: neuroprotection ■ treatment

Ischemic brain injury is triggered by vascular occlusion, either in situ thrombosis or embolization of a clot from a proximal arterial or cardiac source. The vascular occlusion initiates a complex cascade of cellular events, encompassing many different pathways, that ultimately leads to irreversible tissue injury, ie, infarction. The extent and temporal evolution of ischemic injury is influenced by many factors that include the adequacy of collateral blood flow, temperature, glucose levels, and other metabolic factors, and the variability of these factors affects the time window available for initiation of therapy that might ameliorate the size of the ultimate infarction among individual patients. One successful treatment strategy for salvaging ischemic tissue and improving functional outcome after ischemic stroke is reperfusion, as exemplified by the results of the NINDS tPA trial and more recently the ECASS-III trial that has extended the time window for the use of IV tissue plasminogen activator (tPA) out to 4.5 hours in selected patients. The other potential approach to acute stroke treatment is to try to impede the ischemic cascade by targeting various components of the cascade that are deemed to be of importance. This latter approach is called the neuroprotection strategy. Many different neuroprotection approaches targeting different aspects of the ischemic cascade were tested previously in animal stroke models and clinical development programs. Despite many successful treatment experiments in animals regarding both infarct size reduction and improved functional outcome, no neuroprotective drug demonstrated unequivocal efficacy in clinical trials that fulfilled regulatory requirements for approval.

Many reasons for the failure of prior neuroprotective drug development programs were suggested. On the preclinical side, the adequacy and rigor of experimental testing for many drugs was questioned. These concerns led to the STAIR recommendations for preclinical evaluation of purported neuroprotective drugs in 1999 that were recently updated (Table 1). Despite these widely regarded recommendations, many drugs evaluated subsequent to the STAIR proposals failed to meet the majority of the standards suggested. Once neuroprotective drugs were put into clinical trials, many of the development programs were implemented with substantial flaws, as outlined in Table 2, that made adequate evaluation of the potential neuroprotective approach unlikely to demonstrate benefit. This combination of, in many cases, inadequate preclinical testing and flawed clinical trial evaluation for drugs that are likely to be substantially less potent than a thrombolytic such as tPA in demonstrating clinical benefit on outcome measures, such as the modified Rankin scale at 90 days after stroke onset, led to the current pessimism that neuroprotection is not likely to be an effective treatment strategy for acute ischemic stroke. Pessimism about the future has occurred despite the likelihood that none of the prior neuroprotection drug development efforts was performed in a manner that maximized the chances for success. Yet, many neuroprotective drugs have robust and reproducible treatment effects in animal stroke models, with a reasonable therapeutic time window. The question going forward is whether we can translate these effects observed in animals into humans.

Traditionally, the pathway for development and evaluation of a neuroprotective drug was to develop a molecule targeted to impede one aspect of the ischemic cascade, such as glutamate-induced excitotoxicity, free radical–mediated injury, or inflammatory mechanisms, and then perform a series...
of more or less comprehensive animal treatment and toxicity experiments. Drugs determined to reduce infarct size and improve functional outcome with a reasonable safety profile were then brought forward into clinical development, following the usual path of phase I, II, and III clinical trials. Unfortunately, because of the inherent complexities of acute ischemic stroke, the lack of a proven biomarker, and small sample sizes, phase II trials were not informative in most instances regarding supporting efficacy nor for dose-finding. The phase III trials were therefore performed without this useful information to guide them and, in general, were underpowered for detecting modest clinical benefits. Until the most recent trials, they were also performed with a time window for enrollment from stroke onset of up to 6 hours or more and did not include imaging-guided, penumbral identification patient selection, likely to be helpful in later time-window stroke trials, be they neuroprotective or thrombolytic. Because of these and other potential concerns, none of the phase III neuroprotective clinical trial programs demonstrated unequivocal efficacy, and no neuroprotective drug is currently available for treating ischemic stroke. If there is to be a future for neuroprotection, it is clear that future development programs must be implemented differently.

The availability of a safe, even modestly beneficial neuroprotective drug with a reasonable therapeutic time window for ischemic stroke would be of substantial importance in many parts of the developed and developing world. In developed countries, many smaller hospitals do not have adequate resources or personnel to effectively implement IV tPA, let alone intraarterial (IA) therapies, as exemplified by the recent report that 64% of hospitals in the United States did not give tPA between 2004 to 2007. In the developing world, these deficiencies are magnified, so the availability of an effective neuroprotective drug would be of substantial benefit to a large number of stroke patients. The development of a monotherapy neuroprotective drug is currently problematic in developed countries because of the increasing use of IV tPA now recommended out to 4.5 hours after stroke onset and IA therapies in advanced centers. These centers are precisely the ones that usually participate in clinical stroke trials, so organizing clinical trials of new neuroprotective agents that have robust preclinical efficacy and safety data, as suggested by the 2009 STAIR recommendations, will be challenging. One way to develop such a drug would be to organize trials that include centers not doing IA therapy but that have the capability of doing penumbral imaging with MRI or perfusion CT, so that patients not qualifying for tPA (eg, those receiving warfarin or beyond the 4.5-hour window for IV tPA) could be enrolled. Another approach would be to include high-quality centers in developing countries in Asia and South America, where tPA is infrequently or never used. Such trials will be challenging for many reasons, but they could be organized and hopefully well run with the investment of resources and the involvement of centers not usually considered for acute stroke trials. Another consideration for future monotherapy neuroprotective development is the effects of the drug to be tested on the ischemic cascade. In the past, most neuroprotective drugs had very specific therapeutic targets, such as competitive and noncompetitive receptor antagonists. This may be appealing from the pharmacological perspective, but the evaluation of drugs with multiple effects on the ischemic cascade may be more effective in reducing infarct size and improving outcome because the ischemic cascade is diverse and it is likely that many different mechanisms of ischemia induced cell death occur simultaneously. Therefore, the development of neuroprotective drugs with multiple effects on the ischemic cascade is potentially more appealing than drugs acting on only one component of the cascade, if the safety profile is reasonable and the preclinical assessment package fulfills recent recommendations.

Aside from the traditional stand-alone development of a traditional neuroprotective drug, other potential uses for neuroprotective drugs could be considered. Three such possibilities include: (1) neuroprotection as an extender of penumbral survival, so that more penumbra is available for reperfusion therapy; (2) neuroprotection to reduce the conse-

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**Table 1. Initial and Updated STAIR Preclinical Recommendations**

1. Adequate dose–response curve defined
2. Document that the drug accesses the target organ, the brain
3. Define the therapeutic time window in well-characterized animal stroke models
4. All animal treatment experiments should be done in a blinded, randomized manner with control of physiological variables with predefined inclusion/exclusion criteria using an adequate sample size based on an appropriate sample size estimate
5. Both histological and functional outcomes should be assessed acutely and long term
6. Efficacy studies should be performed initially in young healthy male animals using permanent occlusion modeling in most cases
7. Initial studies should be performed in rodents and then studies in gyrencephalic species should be considered
8. Additional studies with promising agents should be performed in female animals, aged animals, and animals with comorbid conditions such as hypertension, diabetes, and hypercholesterolemia
9. Relevant biomarker endpoints such as diffusion/perfusion MRI and serum tissue injury markers should be considered
10. Interaction studies with commonly used medications should be performed

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**Table 2. Some Problems With Prior Acute Neuroprotection Drug Development Programs**

1. Drugs were tested too late after stroke onset
2. Inadequate sample size to detect a modest treatment effect
3. Studies included too many mild or severe stroke patients in whom detecting a treatment effect was difficult
4. Side effects precluded dosing to achieve adequate plasma drug concentrations
5. The study included a substantial percentage of lacunar stroke patients for a drug with no preclinical evidence of protection of the white matter
6. Lack of penumbral imaging in patients enrolled at later time points after stroke onset
7. Inclusion of a substantial percentage of patients who received IV tPA in whom measuring an additional treatment effect is difficult or where the study agent adversely affected outcome with concomitant tPA use

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*Updated recommendations are in bold.*
quences of reperfusion injury after successful recanalization; and (3) developing drugs that have both neuroprotective and recovery-enhancing qualities. The first novel approach of using neuroprotection is to extend penumbral survival, so that reperfusion therapies can be used later and with greater availability of the target of acute stroke therapy, the ischemic penumbra, was evaluated in animals models. In our laboratory, we demonstrated that high-flow normobaric 100% oxygen prolonged the survival of the diffusion/perfusion mismatch on MRI and extended the time window for the successful use of IV tPA as compared with animals who breathed room air. Additionally, we also demonstrated that the purported neuroprotectant, granulocyte colony-stimulating factor (GCSF) also markedly impeded the growth of the diffusion imaging identified ischemic lesion and reduced infarct size on histology. The current randomized FAST-MAG trial of ambulance-initiated magnesium therapy at very early time points after stroke onset may also be testing this hypothesis because many of the enrolled patients subsequently receive IV tPA or IA therapy. If the combination of magnesium and subsequent reperfusion therapy is shown to be superior to reperfusion therapy alone, it may be because magnesium preserved the ischemic penumbra to some extent, allowing the reperfusion therapy to work on a larger amount of target tissue. In addition to testing this hypothesis, the FAST-MAG trial is also of great importance because it is evaluating the concept that neuroprotection initiated within 2 hours of stroke onset could be effective by itself. This latter conjecture may be difficult to determine because the sample size of patients receiving magnesium or vehicle alone is likely to be inadequate to adequately test the hypothesis that magnesium is neuroprotective by itself.

Theoretical concerns that successful and timely reperfusion of ischemic brain might induce secondary injury by mechanisms such as free radical generation, the recruitment of larger numbers of inflammatory cells, or other mechanisms has arisen and been seen to affect the blood brain barrier. This concept of reperfusion injury is supported by observations in both animal stroke models and patients that the initial reversal of diffusion-weighted MRI abnormalities by reperfusion can, in part, have secondary diffusion lesions that subsequently develop. The contribution of such markers of ischemic injury in reperfused tissue that initially appeared to be salvageable to clinical deficits is uncertain, but, as in other vascular beds, the potential deleterious effects of delayed tissue injury after initial improvement secondary to reperfusion injury should be considered. Drugs targeting free radical generation/scavenging and inflammatory white blood cells, for example, could be combined with IV tPA and compared with such treatment alone to determine whether the combination provides additional improvement in clinical outcome on measures such as the modified Rankin scale measured at 90 days. Such combination trials will be challenging to perform, but recruitment at centers actively treating stroke patients with IV tPA should be straightforward, especially because there should be adequate time to explain to patients and families the nature of the trial and potential risks and benefits, so that informed consent can be obtained. In such a trial, vascular imaging with perfusion MRI and MR angiography or CT angiography and perfusion CT should be used to only enroll patients who demonstrate some degree of reperfusion, because patients without reperfusion are not likely to benefit from a drug targeted at reperfusion injury. The major difficulty likely to be encountered in clinical trials evaluating drugs potentially ameliorating the consequences of reperfusion injury will be the need for a large sample size to detect greater clinical outcome than that induced by the powerful effect induced by tPA alone. The use of imaging to corroborate the presence of reperfusion should help in reducing the sample size required to have an adequately powered trial and its use strongly encouraged.

A third novel approach to consider for future neuroprotective drug development is to evaluate drugs with both neuroprotective and recovery-enhancing properties. Such a drug would demonstrate robust effects on reducing infarct size and improving functional outcome when initiated shortly after stroke onset in animals, fulfilling the recently updated STAIR recommendations for both efficacy and safety. In addition, a drug with a dual effect that also includes enhancing recovery should have animal data demonstrating an improvement in functional outcome determined many days or a few weeks after stroke onset that is independent of its neuroprotective effects. For example, if the candidate drug with dual effects is administered at a late time point after stroke onset in an animal model, such as at 24 to 48 hours, no reduction of infarct size will occur, and if functional outcome is improved, it can be reasonably concluded that the observed benefit is related to enhancement of endogenous recovery mechanisms. Such experimental data of tissue salvage when a drug is given early after experimental stroke onset and improved recovery without a reduction of infarct volume when the drug is initiated later are available. Both citicoline and GCSF, 2 drugs currently in advanced clinical trials have robust preclinical data that confirm early neuroprotection and late recovery enhancement effects. This dual mechanism approach combining neuroprotection and recovery enhancement is appealing for several reasons. The therapeutic time window for such drugs should be substantially longer than for drugs that are only neuroprotective because the recovery enhancing effects that contribute to therapeutic efficacy measured at day 90 after stroke onset should be inducible many hours after stroke, as compared with the neuroprotective effect that has a limited time window related to the temporal survival of the ischemic penumbra. This could allow for a relatively late enrollment of patients into the clinical trial. However, if the drug is initiated early after stroke onset, the possibility of both reducing infarct size and enhancing recovery exists, and this potential dual effect could translate into a more robust treatment effect on standard outcome measures such as the modified Rankin scale. Therefore, clinical trials should try to enroll patients within a reasonable time period after stroke onset or possibly use penumbral imaging to stratify patients into those with or without a substantial penumbra to determine whether one group is more likely to have a better response to the treatment being evaluated. When the results of the current citicoline and GCSF trials become available, we will learn whether the dual
mechanism approach will work and also how to design better trials of such agents in the future.

The concept of neuroprotection for treating acute ischemic stroke is scientifically sound but one that has not yet translated into a therapy with clear proof of efficacy in the clinical setting, despite the investment of substantial resources by industry, academia, and governmental granting agencies. It is likely that neuroprotection can be shown to have a significant treatment effect, either as a monotherapy or in combination with pharmacological or mechanical reperfusion. The challenges going forward to provide conclusive proof of such efficacy are many, as are the lessons to be learned from past drug development programs. Neuroprotection should not be abandoned because, ultimately, the treatment of acute ischemic stroke will require not only fixing the plumbing but also impeding the tissue and cellular consequences induced by the vascular occlusion and its removal. The stroke research community should support the development of novel neuroprotective agents in carefully designed and conducted preclinical and clinical development programs that try new approaches to demonstrate efficacy as suggested previously or that will be suggested by other members of this community.

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