Neuroprotection

Challenges and Opportunities

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The ability of pharmacological agents to limit secondary biochemical damage and cell death has been well established in numerous animal models of stroke, head injury, and spinal cord injury, yet the results of such neuroprotective treatment strategies in human injury have been disappointing. A number of conceptual and methodological issues have undoubtedly contributed to the difficulties in translating the experimental results to the clinic. Most recently, different experimental approaches and altered clinical trial methodologies have provided renewed, albeit cautious, optimism regarding future clinical trials of neuroprotective agents. Herein, we reviewed both the critical problems and potential solutions, emphasizing recent experimental and clinical work.

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During the past 25 years, animal models of central nervous system (CNS) injuries have provided substantial experimental support for the concept that delayed biochemical damage contributes to tissue loss and chronic behavioral dysfunction. Such studies have also been the basis for the development of neuroprotective treatment strategies. However, after numerous failed clinical trials of drugs showing preclinical promise (Table), questions have been raised about the relevance of current experimental models for human brain or spinal cord injury. This has reduced enthusiasm for clinical trials of neuroprotection, particularly within the pharmaceutical industry. Moreover, these failures have underscored the translational difficulties in moving such research from bench to bedside. Critical preclinical issues include the clinical predictiveness/relevance of animal models, the adequacy of pharmacological methodology, and outcome measures used. Clinical concerns have included issues of sample size, injury severity, treatment time, and statistical analysis, among others.

Despite this challenging experimental environment, recent advances in both experimental approach and trial design have provided a basis for cautious optimism. Experimental drug studies have more consistently addressed such questions as therapeutic window, dose-response profile, and CNS penetration. Multipotential drugs that serve to target multiple components of the secondary injury cascade have been developed or identified. Other compounds that serve to activate or up-regulate endogenous neuroprotective pathways have also been identified. Establishment of clinical consortia with prior extensive trial experience has led to improved consistency and consensus across centers. Other clinical changes in trial design have included increasing sample size, targeting moderate injury levels, improving statistical methodology, and identifying more appropriate subgroups. Our review addresses these various challenges and opportunities.

PRECLINICAL CHALLENGES

Numerous methodological problems have been raised with regard to development of animal models that have meaningful clinical relevance. One area of con-
certain relates to the choice of species, strain, or sex of the animal. For example, how well do models of brain trauma or ischemia in rodents reflect injury in higher species? Moreover, even within a given species, the same model can produce vastly different injury levels and outcomes across various strains. This issue is critical both for evaluating drug targets and regarding the choice of outcomes. Thus, identical brain contusion parameters in SV129, C57B, or FVB mice induce mild, moderate, or severe injury, respectively, with only moderate injury across models, making it difficult to even use certain outcome measures in particular strains. Few studies have compared effects of treatment in male vs female animals; however, females appear more resistant to injury in certain animal models, as well as in human studies. Although traumatic injuries to the brain or spinal cord occur most frequently in males in their late teens and early 20s, many experimental studies, particularly in spinal injury, are conducted in female animals; in part, this reflects the greater difficulty of postinjury bladder expression in male rodents. Each of these issues poses potential translational difficulties.

With very few exceptions, animal models of traumatic or ischemic injury use anesthetized preparations. However, various anesthetics affect injury in different ways and may serve to enhance or reduce cell death as a function of developmental age, as well as have considerable effect on drug actions. This creates potential problems in translating experimental observations to unanesthetized humans.

The vast majority of preclinical neuroprotection studies, including most that have served as the basis for subsequent clinical trials, have been inadequate with regards to pharmacological evaluation. These inadequacies include a lack of complete dose response, a failure to examine therapeutic windows or pharmacodynamic, or an absence of pharmacokinetic, pharmacodynamic, or brain penetration data. Had such data been available, initiation of certain negative clinical trials might have been prevented, possibly resulting in better designed studies.

Animal trial design and statistical analyses also differ considerably from those used in human trials. Whereas clinical studies generally employ an intent-to-treat analysis, this is virtually never done preclinically. Rather, if a drug is administered in error or at the wrong dose or if injury seems inadequate, animals are usually dropped from the study and replaced. Power analysis is not generally performed to determine optimal animal sample size; rather, the number of animals is often determined either arbitrarily (on the basis of historical data with other drugs), or, worse, the sample size may be increased incrementally during the study (if there are treatment trends) until statistical differences are achieved. Many laboratories also delete outlier data. Although this can be done legitimately as part of the initial trial design, such deletions are not uncommonly performed post hoc and without sufficient rigor or consistency.

Lastly, most animal models are highly constrained. To increase consistency, genetically identical animals are used under highly controlled experimental conditions. Therefore, brain trauma models usually examine effects of local contusion or diffuse axonal injury in models that do not include significant ischemia, hypoxia, mass effects, or associated systemic injuries, as in human trials. Although the more limited and focused animal studies are justified by concerns about time and cost, profound differences from human traumatic brain injury may create serious translational difficulties for drug studies. This is-

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<th>Drug Class</th>
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<td></td>
<td>Clinical</td>
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<tr>
<td>Anti-inflammatory</td>
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<td>NMDA antagonists</td>
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<td>AMPA antagonists</td>
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<td>Dexabalin</td>
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<td>Growth factors</td>
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<td>Glucocorticoids</td>
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<td>Caffeine</td>
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<td>Opioid antagonists</td>
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<td>Free radical scavengers</td>
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<td>Erythropoietin</td>
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<td>Calcium channel blockers</td>
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<td>Magnesium sulfate</td>
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<td>Statins</td>
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**Table. Comparison of Preclinical and Clinical Neuroprotective Studies for Selective Drug Classes**

Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; ND, study not done; NMDA, N-methyl-D-aspartate; TRH, thyrotropin-releasing hormone; +, mild protection; +, +, moderate protection; +, +, +, strong protection; ±, some studies showed no protection whereas others suggested protection; –, no protection.
sue also underscores the desirability of designing appropriate stratification into the clinical trial design.

**CLINICAL CHALLENGES**

As indicated previously, there are many methodological issues that have limited the clinical application of therapies that have shown effectiveness in various animal models of acute neurodegeneration. Inadequate preclinical pharmacological evaluation is probably a major factor. Failure to optimize dosing, to demonstrate sufficient CNS penetration, or to show effectiveness with treatment delays common in human studies represent some of the key issues. For example, there have been numerous failed clinical studies of the use of glutamate antagonists in treating stroke or head injury.\(^{10,11}\) Preclinical studies have generally shown effectiveness only under pretreatment or very early posttreatment conditions, which markedly contrasts with the clinical studies. The limited therapeutic time window for these agents may reflect the early wave of glutamate release after injury, the rapid down-regulation of N-methyl D-aspartate receptors after an insult, or the targeting of largely necrotic cell death, which peaks in the first hours after injury. In the National Acute Spinal Cord Injury Study II series, which compared methylprednisolone and naloxone hydrochloride with placebo after spinal cord injury,\(^ {12}\) the chosen dose of naloxone was too high and did not reflect the inverted U-shaped dose-response curve found in animal studies.\(^ {13}\)

In contrast to animal studies, human trials have often included a wide range of injury levels. Inclusion of mild injury may lead to a ceiling effect in the absence of a very large sample size, whereas severe injury may not be amenable to pharmacological intervention. Although use of more carefully chosen subgroups may extend the length of a study, it may serve to reduce the likelihood of a negative result. Consistent with this conclusion, participants in the National Acute Spinal Cord Injury Study II series showed significant, albeit modest, neurological improvement at 12 months for both methylprednisolone and naloxone administration though only in the subset of incomplete injured patients treated within 8 hours of trauma.\(^ {12}\)

It has long been recognized that many of the earlier neuroprotection trials were underpowered. In the recent Corticosteroid Randomisation After Significant Head Injury trials, sample size was markedly increased to nearly 10,000 patients to detect a minimal but significant therapeutic response.\(^ {14}\) Although such a number may be excessively large, it may enable discrimination of only a few percentage points of improvement with therapy, and it underscores the recognition that many prior major trials were powered only to detect relatively marked treatment effects. It must also be appreciated that major improvements in early supportive management of patients, including prehospital care and development of specialized treatment units, have improved long-term recovery of patients with head or spinal cord injury.\(^ {15,16}\) This makes it more difficult for clinical trials to show significance because of ceiling effects, amplifying the need for greater sample sizes.

**OPPORTUNITIES**

**Multipotential Drugs**

A multitude of animal studies across models and species have demonstrated that secondary injury involves multifactorial biochemical processes initiated within minutes to days or longer after the insult.\(^ {4}\) Such processes involve interactive relationships among neurons, glia, and microglia (Figure 1), as well as complex and highly interactive signal transduction cascades (Figure 2). For example, activation of microglia amplifies neuronal damage through secretion of cytokines, such as IL-1β (interleukin 1β) and tumor necrosis factor α, as well as by increasing production of reactive oxygen species and nitric oxide. However, they may also result in neuroprotection by secreting growth factors, such as tumor necrosis factor β. Activation of astrocytes also enhances generation of reactive oxygen species and nitric oxide and inhibits neuroplasticity by generating axon-inhibitory ligands, such as chondroitin sulfate proteoglycans. Glutamate released from neurons may also contribute to the formation of reactive astrocytes. In contrast, astrocytes also have a neuroprotective function by removing glutamate from the microenvironment. Examples of treatment strategies that attenuate microglial and astrocyte responses causing neuronal cell death are illustrated (Figure 1). Unfortunately, most of the experimental work that has led to clinical trials has been based on the modulation of a single proposed injury factor. Although such treatments can be shown to be beneficial in highly constrained animal models, they are less likely to prove efficacious in the more complex human disorder that involves more variable degrees of injury severity in a genetically diverse population.

Trials in cancer and infectious diseases have shown that for many diseases, multidrug strategies are required for optimal therapeutic outcome. Remarkably, few experimental studies of acute neurodegeneration have examined multiple drug treatments, and such an approach is not practical in humans given the high cost of neuroprotection trials and the limited effectiveness of agents that have already been studied. Instead, a number of laboratories have developed or evaluated single compounds that have multipotential effects on multiple identified injury mechanisms (Figure 2). The first such examined agents have been naturally occurring hormones, such as corticosteroids, thyrotropin-releasing hormone, and progesterone, or their synthetic analogues. For example, methylprednisolone has both anti-inflammatory and antioxidant effects, among others.\(^ {12}\) Although corticosteroids have not proved effective in human head injury or stroke,\(^ {10,14}\) a modest yet significant neuroprotective effect has been reported in a subgroup of patients in a phase 3 trial of spinal cord injury.\(^ {12}\) Thyrotropin-releasing hormone has multiple reported neuroprotective actions, including enhancing cerebral blood flow and metabolism and serving as a physiological antagonist of peptidyl leukotrienes, platelet-activating factor, endogenous opioids, and possibly glutamate.\(^ {17}\) Many experimental studies of brain and spinal cord injury across models and species have reported significant neuroprotective actions of thyrotropin-releasing hormone or its
analogue,17 and a small pilot clinical study of spinal cord injuries showed significant beneficial actions.18 Remarkably, although this work dates back to 1981, no large randomized clinical trial investigating the neuroprotective properties of thyrotropin-releasing hormone has been conducted. Several groups have reported neuroprotective actions for progesterone in traumatic injuries in preclinical models and have suggested that the effects may result from its multipotential actions.19,20

More recently, a number of other multipotential compounds have been synthesized, and these show strong neuroprotective actions in vitro and in vivo. A class of novel cyclic dipeptides (diketopiperazines), structurally similar to a physiologically active metabolic product of thyrotropin-releasing hormone, have shown neuroprotection in neuronal cell cultures against oxygen and/or glucose deprivation, mechanical injury, excitotoxicity, free radical damage, and various proapoptotic and pronecrotic insults, also markedly reducing lesion volume and improving motor and cognitive outcomes across multiple species and models of brain injury.17,21 These diketopiperazines modulate gene regulation, downregulating factors that promote cell death (such as cell cycle proteins, cathepsin, and calpains) while upregulating endogenous neuroprotective pathways (such as heat shock proteins, brain-derived neurotrophic factor, and hypoxia-inducible factor-1).21 AM-36 is a sodium channel blocker with an antioxidant moiety that has diverse neuroprotective actions both in vitro and in vivo.22 Neuroprotective effects of erythropoietin have been shown in animal models of stroke, head injury, and spinal cord injury and have been proposed to reflect multifactorial actions.23 Protective actions are also found with carbamylated derivatives that lack erythropoietic activity.24 Quite recently, propargylamine monoamine oxidase inhibitors have been developed; they have shown neuroprotective actions against multiple forms of neuronal injury in vitro, inhibiting apoptotic mechanisms and modulating various microtubule-associated protein kinases and cytokines.25 Collectively, these studies demonstrate the potential of multipotential treatment strategies.26 However, substantial hurdles remain. Two other promising experimental drug treatments with multipotential actions, which have shown effectiveness across experimental models, have recently failed in randomized clinical trials of head injury: the synthetic cannabinoid dexanabinol and magnesium sulfate.27,28 Such studies highlight the important caveat that even strong experimental data in lower species may not predict therapeutic effectiveness in human injury.

Figure 1. Complex relationships exist among neurons, astrocytes, and microglia after central nervous system injury. The red arrows indicate interactions contributing to neuronal death, whereas mixed-color arrows indicate interactions that may be either positive (neuroprotection, green) or negative (cell death, red). NO indicates nitric oxide; ROS, reactive oxygen species.
Facilitating Endogenous Neuroprotective Pathways

It is well established experimentally that a variety of non-pharmacological manipulations may provide neuroprotection. These include exercise, caloric restriction, and certain kinds of mental activity or environmental enrichment. It has been proposed that these diverse stimuli serve as environmental stressors, which activate endogenous neuroprotective factors (such as brain-derived neurotrophic factor and heat shock proteins), similar to what occurs with preconditioning. Collectively, these observations suggest the possibility of identifying or developing drugs that activate these same endogenous neuroprotective pathways. Such compounds may include certain antidepressants, statins, and diketopiperazines. Consistent with this theory, it has been shown that structurally diverse compounds that up-regulate heat shock proteins, which can serve to limit both necrotic and diverse apoptotic pathways, provide considerable neuroprotection in animal models of stroke. Administration of various neurotrophic factors has also demonstrated neuroprotection in experimental stroke, head injury, and spinal cord injury models but have failed in clinical trials, possibly reflecting poor penetration through the blood-brain barrier.

Genomics and Proteomics

Numerous studies that use advanced genomics tools to identify potential endogenous neurotoxic or neuroprotective pathways after CNS injury have been published. In some cases, these have led to the testing of new neuroprotective strategies in experimental models. For example, identifying up-regulated cell cycle gene pathways associated with neuronal cell death, gliosis, and microglial activation led to highly effective treatment with cell cycle inhibitors in rodent models of brain injury. Moreover, identification of gene clusters associated with chronic inflammation and microglial activation after CNS injury may explain the late neurodegeneration that occurs after CNS trauma and possibly the epidemiological relationship between head injury and subsequent chronic neurodegenerative disorders, such as Alzheimer disease or Parkinson disease. Subacute targeting of such inflammatory pathways may prevent such delayed or chronic neurodegenerative processes.
Although less work has been performed regarding proteomic approaches to identify novel neuroprotective targets (in part because of the increased complexity and expense involved), proof-of-principle studies have been published. As functionally relevant analysis of such large data sets becomes more feasible, it appears likely that genomic, proteomic, and possibly metabolomics studies may lead to identification of additional or perhaps better treatment options.

Opportunities in Clinical Trials Design

Multicenter consortia have been developed in a variety of areas to increase the number of patients and to address issues such as intercenter variability, population selection, and standardizing treatment. Among the major differences between animal and human studies of neuroprotection is choice of outcome measures. Most preclinical stroke studies use infarct volume as a primary outcome. In contrast, given the poor correlation between infarct volume and behavioral outcome in humans, clinical studies have preferentially used behavioral outcomes, such as death or disability. Identification and use of appropriate surrogate end points could provide a substantial advance for the evaluation of acute neuroprotective strategies. Using Bayesian statistical methodology may serve to improve the efficiency of trial design by facilitating identification of optimal dosing and reducing sample size requirements. Better patient selection may help to match patient populations with proposed therapeutic targets. Finally, clearer delineation of the requirements for establishing and optimizing neuroprotection in animal models should be developed and met before drug candidates are moved into the clinic. The Stroke Therapy Academic Industrial Roundtable group has proposed such criteria and should serve as a model to develop translational criteria for neuroprotection in other disorders. One drug that has met the stringent preclinical Stroke Therapy Academic Industrial Roundtable criteria is NXY-059, a free radical trapping agent with the ability to modulate apoptotic factors. Although preliminary analysis of a recently completed phase 3 trial in patients with stroke has suggested significant improvement in one behavioral end point, concerns have been raised about the statistical analysis in that study. These reports underscore both the potential and difficulty of establishing clinical efficacy for new neuroprotective agents.

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REFERENCES


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