Novel Multi-Modal Strategies to Promote Brain and Spinal Cord Injury Recovery

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Abstract—Stroke is the leading cause of disability in the United States, and yet no definitive interventions can drive the nervous system beyond its measurable but often limited spontaneous recovery. Treatment to limit injury progression and enhance repair after stroke or other types of central nervous system injury is complicated by the heterogeneous nature of cell death and wound healing mechanisms and the multiple barriers to functional recovery. The heterogeneity of injury and repair mechanisms requires interventions that are broad and multi-modal, but also intrinsically safe. We describe a process to identify such interventions by screening multiple individual targets in the historically separate realms of neuroprotection, repair, and regeneration against a library of FDA-approved compounds with known safety. We have identified nearly 10 compounds that are able to activate simultaneously protective and reparative genes. These compounds have a theoretical therapeutic window that spans from evolving injury (minutes to hours) to stable injury (days to months to years). It is our hypothesis that these compounds will be most efficacious when paired with training. The notion is that drugs will alter the propensity of the nervous system toward recovery, whereas specific training will engage the needed instructive cues to achieve this goal. Indeed, robotic training can provide a level of motor learning that seems to enhance the salutary effects of training. In a system that depends heavily on cells that do not easily replenish themselves, cellular therapies could also ultimately be an important part of the cocktail. We conclude that combinations of interventions will be needed to surmount the multiple barriers to recovery in stroke and other types of brain and spinal cord injury recovery. (Stroke. 2009;40[suppl 1]:S130-S132.)

Key Words: brain recovery ■ spinal cord recovery ■ treatment

Despite its prevalence and heavy societal toll, very few prospective clinical trials are ongoing in the US to limit disability after stroke.1–3 The lack of human studies is particularly remarkable given the enormous explosion in our understanding of the biological basis of neural protection and regeneration in animals.4 For too long, we have trusted that these preclinical biomedical advances would “trickle” down into better treatments for our patients. We are gradually realizing that this is not a reasonable assumption and positive steps must be taken to reverse this unsuccessful strategy.18

Infrastructures must be developed that facilitate the movement of inspiring biological advances related to stroke recovery to their safe and rigorous examination at the human bedside. These infrastructures must draw on wide expertise.5 The multi-disciplinary nature of stroke recovery research has evolved from a recognition that a single “magic bullet” will unlikely provide all the instructive cues necessary to completely or partially recapitulate the development of a “new nervous system,” including promoting the birth of new neurons, increasing axon growth, enforcing the formation of new synapses, and providing a normal glial environment.6 Moreover, stroke comprises a large group of distinct phenomenologies from lacunar infarction to large vessel occlusion to subarachnoid hemorrhage.7 Together, these observations suggest that any successful intervention in neurorehabilitation will involve a multi-modality approach.

Another potential failure of current attempts to develop new therapeutic strategies for the treatment of stroke is the inability to functionally distinguish between those aspects of progression that represent distinct biological epochs of stroke from those that may be part of a therapeutic continuum that may be accessible by similar therapeutic approaches.8 Thus, while there is no doubt that there are interventions that provide events only if applied at specific time points after injury (eg, tissue plasminogen activator), it is also critical to develop an additional conceptual framework that views the biology in evolving and stable stroke as separable and broad, but interrelated. An extension of this biological “connectedness” is the notion that it will be possible to develop therapies that are effective in multiple biological epochs after stroke onset. Of course, a first priority of neurorehabilitation is to prevent brain injury.9 Targets to accomplish this goal include agents that interdict ischemia-related cell death pathways10,11 or stimulate adaption to hypoxia or hypoglycemia. Included
in the broad area of “neuroprotection” are also agents that minimize deleterious inflammatory responses. However, for the most part, inflammation is an adaptive response to injury and it is engaged to promote wound healing and repair. It marshals the onset of a second recognizable biological epoch after injury that includes the destruction of myelin and the formation of a chemical and cellular barrier known as the glial scar. Myelin and the glial scar provide separable but likely synergistic barriers to regeneration in the central nervous system. In a third epoch, the brain must find ways to stimulate remyelination, neurogenesis, axon extension, and synaptogenesis, and to penetrate unfriendly myelin and glycosaminoglycans. Of course, aberrant sprouting and synaptogenesis can cause well-known complications such as pain and spasticity.

We have identified small molecule “drugs” that target all interleaving biological epochs poststroke. Moderate to high throughput assays have been developed to screen for small molecules that enhance survival pathways, diminish death pathways, overcome glial inhibition, suppress the glial scar, enhance axon growth, stimulate neurogenesis, and promote axon extension. We have attended to a number of details that create variability between screens: concentrations of drug used, methods of storage and solubilization, endpoints, library, and so on. We initially used a 2000-compound library assembled by Microsource Discovery, Inc, that includes FDA-approved drugs, nutriceuticals, and some reference chemicals. As the salutary and toxic effects of each compound on each target emerged from each of the screens, they were inputted into a computer to facilitate bioinformatics analysis. The rationale here was to prioritize compounds based on their ability to manipulate target activity in the direction of protection and repair and ultimately to try select compounds that had shown efficacy in several or all of the screens. Those that were positive in multiple screens also had to pass the test of not being toxic in screens where they are not positive. Interestingly, out of 2000 compounds, almost 7 met these criteria. Some of these compounds are nutriceuticals; others are used regularly in the medical pharmacopeia.

Are our 7 multi-target “hits” simply dirty drugs that raise the concern that some unknown target of action will doom them as they move through testing to clinical trials? The answer is maybe, but hope lies in our biological strategy for choosing targets. This strategy relies heavily on the concept of homeostasis. Homeostasis refers to the ability of a stressed organism to mobilize defenses that allow it to return to a set point that is consistent with survival. Adaptive stress responses to hypoxia–ischemia involve the activation of preexisting proteins as well as de novo gene expression. The posttranscriptional and transcriptional adaptive responses involve >70 genes and their corresponding proteins. These proteins can act at a cellular, local, and systemic level to alleviate the stress associated with ischemia. In some circumstances, this means converting the nervous system in the short-term to anaerobic glycolysis; in others, it means deleting dysfunctional neurons via apoptosis. Our model suggests that small molecules that enhance adaptive responses to ischemia and inflammation will include protective and reparative genes.

Finding small molecules that activate or augment programs of adaptive gene expression after stroke is only part of the battle. Once drugs are identified, algorithms must be in place to define the route of administration, vehicle, and dosage that allow the target of interest to be manipulated in the desired fashion in vivo. The relatively recent development of in vivo bioluminescent imaging offers a technically straightforward way to accomplish this goal. Luciferase reporter genes controlled by single response elements of large noncoding regions of genes of interest can be expressed in cell types of the central nervous system of transgenic mice or in native mice using viral vectors. A drug is then delivered by the desired route and, at various times afterward, the rodent can be fed D-luciferin. If the reporter is induced by the drug of interest, luciferase is expressed along with it substrate and light is generated. As the emission of luciferase is in the near infrared, light passes freely through tissue, including the skull, without absorption. These methods not only allow efficient and technically feasible ways for determining whether a drug or its metabolites can activate a drug target of interest in the central nervous system but also can determine how long the target is activated. Historically, ideal dosing is determined by highest, safest dose. In vivo bioluminescence imaging identifies the dose that is most effective at activating the target of interest. Given that dose responses are parabolic or worse, defining dose responses based on activity has obvious advantages.

The burgeoning interest in stem cell research is partly explained by the recognition that in a tissue, such as the brain where cells are permanently lost, even with the best drug function it cannot be optimally restored unless cells are replaced. The remarkable pace of progress in the field of stem cell science offers multiple opportunities to develop cell-based interventions that may offer new opportunities to provide benefit to stroke patients.

In considering cell-based therapies, it is necessary to determine what cells have been damaged, what cell (or cells) are optimal for use restoring in normal tissue function, and what opportunities exist for actually worsening the plight of the stroke patients. Each of these may represent formidable undertakings, but in each of these areas significant progress is being made.

One of the major challenges in considering cell replacement therapy for stroke is the number of cell types that may be damaged after such injury. Both projection neurons and local interneurons may need to be replaced, as well as myelin-forming oligodendrocytes, the astrocytes that provide multiple forms of metabolic and trophic support to other cells of the central nervous system, and also vascular cells themselves.

Recent studies from the field of spinal cord injury raise the prospect that astrocyte transplantation therapy may represent a particularly attractive starting point for repair of central nervous system damage. In these studies, glial-restricted precursor cells derived from the embryonic spinal cord were expanded in vitro and induced to differentiate into astrocytes (by exposure to bone morphogenetic protein–4) before transplantation.

Studies on astrocyte transplantation therapy also have provided cautionary information about the possibility of doing harm by cell transplantation, with findings that demonstrate how critical a challenge it is to identify optimal cells for performing repair. Although the generation of pain
syndromes seems unlikely to be of concern in treatment of stroke, the question of whether cells being transplanted may actually inhibit ongoing repair processes needs to be much more carefully examined than has been the case to date. Despite the promise of drugs and cellular replacement, the regulatory sequencing required for proper optimal recovery of function (eg, axon targeting, synapse formation, and so on) after stroke transcends each of these interventions. A therapeutic strategy that holds promise in this realm is robotic training. Robots have been developed that facilitate appropriate movement and discourage aberrant movement. In encouraging appropriate movement, the robot creates “spongy,” virtual walls. Narrower distances between the walls provides less room for error, while wider distances provide greater room for error. The wider the walls, the more difficult it is for the patient. Overcoming this difficulty to create linear vectorial movement from the center of screen to a point in the periphery engages “motor” learning. The process of engaging motor learning seems to create an avenue for recovery of impairment in patients with stable injuries many years out from the ictus that is superior to standard rehabilitation. Optimal robotic training and its mechanisms of action are still being explored.

Our aspiration is to ultimately combine drugs or cells with robot training as a strategy for stimulating beneficial changes in impairment, disability, and handicaps. The identification of compounds with known safety in humans with protective and reparative capacity and the development of effective cell-based therapies provide hope that multimodality human clinical trials involving drugs, cells, and robots for evolving or stable stroke recovery is doable in the near term. Following the lead of many Cancer Institutes and Hospitals, it our belief that every patient in a rehabilitation setting with a poor prognosis for functional recovery should be entered into a clinical trial. Building such infrastructures is critical to improving quality of life for the millions of individuals throughout the world disabled because of stroke and other disabling injuries such as traumatic brain injury and spinal cord injury.

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References
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