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Confirming an experimental therapy prior to transfer to humans: What is the ideal?

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Abstract—As the spinal cord injury (SCI) scientific community moves closer to translating experimental data to the clinic, specific steps should be addressed to improve our chances of success. Some of the steps under discussion include animal modeling, clinically relevant endpoints, compelling evidence for improvements, and safety issues. First, it will be beneficial if exciting data are first replicated before findings are considered clinically relevant. Then major findings must be published in peer-reviewed journals so that the scientific community may scrutinize the data. Finally, continued communication between different research groups throughout the world, as well as between basic scientists and clinicians working in the area of SCI, will enhance our progress in this important research field.

Key words: animal models, behavior, electrophysiology, human spinal cord injury (SCI), neuropathology, neuroprotection, outcome measures, replication studies, safety, spinal cord trauma, transplantation.

INTRODUCTION

There has never been more optimism in the scientific community that novel treatments targeting spinal cord injury (SCI) are being discovered that will improve neurological function in humans [1,2]. To date, several treatments have been reported to promote a degree of axonal regeneration and functional recovery in the spinal cord [3–14]. Also, several neuroprotective strategies administered in the acute injury setting have shown promise in reducing secondary injury mechanisms and promoting recovery [15–22]. Thus, an important question now being discussed in the scientific community is whether these neuroprotective or regenerative strategies are ready for clinical application [23]. Organizations such as the International Spinal Research Trust (ISRT) have taken the view that any treatment that has the prospect of lowering a human cord injury by two spinal levels (~2 cm) should be considered for human trials. Thus, an obvious question as we refine these potential treatments and move them forward is, what are the ideal conditions on which

Abbreviations: ASNTR = American Society of Neural Transplantation and Repair; BBB = Basso, Beattie, Bresnahan; FDA = Food and Drug Administration; ISRT = International Spinal Research Trust; NIH-NINDS = National Institutes of Health, National Institute of Neurological Disorders and Stroke; SCI = spinal cord injury.

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to base new clinical therapies? In this regard, several relevant questions immediately come to mind, including: What are appropriate animal models to test new treatments? What degree of efficacy should be considered clinically significant? Under what conditions can a laboratory finding best be replicated? What will be the Food and Drug Administration (FDA) and safety requirements? Some basic criteria must be met if new therapies are to be proposed for clinical investigations:

- the therapy works in several animal models;
- the therapeutic window is wide;
- the therapy results in robust improvements in structural and functional outcome;
- the study is clinically relevant, replicated in an independent laboratory;
- improvement is seen in large animals, with clinically relevant endpoints;
- major findings are published; and
- safety issues are addressed.

ANIMAL MODELING ISSUES

To obtain the necessary experimental data to begin clinical studies, compelling evidence for benefit must be demonstrated in reproducible animal models of SCI. Although no single experimental model exactly mimics the clinical condition, animal models allow for the rigorous study of pathomechanisms of injury and recovery. Appropriate rodent models that are currently being investigated include compression, contusion, and transection methods leading to reproducible patterns of structural damage in specific gray- and white-matter structures. With each model, injury severity can be varied so that a spectrum of histopathological and behavioral deficits can be reproduced. It is important to note that the SCI patient population is a very heterogeneous group, with no one SCI being exactly the same as another. For example, varying degrees of white- and gray-matter damage may occur at different or multiple spinal cord levels. This reality of the clinical problem should always be emphasized when trying to model human SCI in experimental investigations.

As indicated by the American Society of Neural Transplantation and Repair (ASNTR), the exact type of animal model that is required will depend on the target condition being considered [24]. Rat models of SCI are the most commonly studied in both neuroprotective and reparative investigations, because of their low cost, the small size of the animal, ease of handling, and established SCI methods. These animals can be anesthetized and intubated so that physiological parameters such as PO_2, PCO_2, pH, and blood pressure can be monitored and maintained in normal ranges. This step is important because many pharmacological treatments have significant effects on physiological variables and may complicate data interpretation unless this information is obtained and reported.

Recently, mouse models of SCI have been developed and investigated. These models are advantageous because genetic factors associated with cell death and axonal regeneration can be rigorously investigated to determine cause-and-effect relationships between gene expression and outcome. The ability to enhance or delete specific genes by transgenic mechanisms is generating important information on how growth and inhibitory factors affect axonal outgrowth. The ability to investigate developmental processes, including axonal guidance molecules and cell death mechanisms, is also an important use of these models.

Only recently has gender been appreciated as a critical factor in determining the vulnerability of central nervous system tissue to injury, as well as influencing therapeutic interventions. Experimental studies in models of stroke and brain trauma, for example, have shown that adult females are resistant to acute injury mechanisms [25–28]. In models of focal brain injury and trauma, female rats have smaller infarcts and contusions when compared with age-matched males [25,26]. Removing circulating hormones such as estrogen and progesterone by ovariectomy lifts this protection and leads to increased damage. Thus, gender considerations are also important when testing preclinical therapeutic SCI interventions. It is interesting to note that most SCI experimental models are produced in female rats, because they experience fewer bladder infections than males. However, it should be stressed that in clinical SCI, the majority of patients are young males. Thus, before a new experimental therapy is moved to the clinical arena, it will be important to demonstrate the benefit in both genders.

Nonhuman primate models of SCI are also considered important in testing experimental therapeutic strategies [29,30]. In addition to various neuroanatomical considerations, the size of the primate spinal cord more closely approximates that of the human specimen. This point is important because invasive surgical procedures may be required to transplant cells, or administer growth

factors or anti-inhibitory molecules into the injured human spinal cord. Large animal models can therefore be helpful when refining these invasive transplantation strategies. Also, outcome measures that closely mimic those in proposed patient studies can be used in nonhuman primate models to quantitatively assess outcome [30]. These include electrophysiological measures for sensorimotor function, as well as locomotive outcome measures.

COMPELLING EVIDENCE OF BENEFIT

Probably the most important factor when discussing the potential for a treatment to be moved to the clinic is the degree of benefit, in terms of established outcome measures. As previously discussed, several strategies have been reported to improve functional outcome in different experimental settings. It is therefore important to consider what these findings would mean to the SCI patient in terms of their disability if they were successfully translated to the clinic. For example, if a therapeutic intervention significantly improves Basso, Beattie, Bresnahan (BBB) locomotor function [31], the significance of this degree of change, in terms of overall motor improvement, must be considered. An important goal of various research groups is the development of new approaches for monitoring physiological and structural changes in patients with SCI. Such assessment tools, including sensory and motor-evoked potentials and high-resolution imaging for documenting lesion progression and the behavior of implanted cells, are not presently available in routine clinical practice. However, these types of assessment tools will play an important role in the advancement of clinical trials for spinal cord interventions.

Other factors relevant to therapeutic interventions, such as dosing, therapeutic windows, and the degree of neuroprotection or regeneration, are extremely important to consider. For example, if a neuroprotective agent is only effective in protecting neurons or white-matter tracts from irreversible damage when given before or immediately after injury, this approach may have severe limitations in the clinical arena, where delayed post-treatment is commonly used. However, it should be mentioned that pretreatment strategies are a reasonable approach for neuroprotection under surgical protocols that can potentially cause paralysis. Also, pretreatment strategies during transplantation surgery could limit the potentially harmful effects of invasive procedures necessary to transplant cells or administer or growth factors.

The number of regenerative axons within or exiting from a graft is used as one assessment of reparative strategies. Commonly, treated tissues are assessed for numbers of axons and compared with appropriate controls to determine whether a specific treatment is beneficial. The length and number of axonal branching points are also considered in anatomical studies as an outcome measure to assess specific interventions. Although these approaches are important to assess potential mechanisms of improved behavioral outcome, they do not necessarily correlate with functional improvement. Likewise, a decrease in the amount of tissue injury with a neuroprotective treatment, while important, does not provide information about whether these structural changes will translate into improving electrophysiological or behavioral outcome. Thus, quantitative methods of assessing outcome after experimental treatments need to be clinically relevant [32,33]. Also, it is hoped that the observed improvements are robust and reproducible from animal to animal. Only then should therapies be considered for human trials.

Quite frequently, it is said or written that “we don’t need to know how it works if it improves outcome following SCI.” Although not a prerequisite for clinical consideration, understanding the basic mechanisms by which a therapy works is considered important by the scientific community. Often, a treatment will affect various mechanisms, some of which are well established. Clarifying these mechanisms may ultimately help with the continued investigation of a specific therapy targeting cell death or axonal regeneration. If a therapy is partially protective or demonstrates a significant but mild improvement of function, knowing the mechanisms for this effect may assist the investigator in revising the treatment protocol and, hopefully, promoting more complete recovery. Understanding the mechanisms of therapy is also critical when cause-and-effect relationships between a treatment and an observed outcome measure are investigated. These types of relationships are critical to scientists attempting to obtain extramural funding for their laboratories as well as improve outcome in injury models of SCI.

REPLICATION STUDIES

Much too frequently, a single study is published that brings excitement to the SCI field, but for various reasons

the finding cannot be replicated. This lack of replication significantly decreases the overall importance of the finding, in terms of potential clinical efficacy. Although the particular study may be well done, with proper controls and adequate description of methods, the publication may lack critical information required for an independent investigative group to exactly replicate the study. Also, there is a general lack of enthusiasm in the scientific community to replicate published data from another laboratory because of a lack of scientific interest, publication concerns, and the constraints of a commitment to granting agencies.

In this regard, the National Institutes of Health, National Institute of Neurological Disorders and Stroke (NIH-NINDS), has recently initiated a contract program to establish a priority list of published studies that merit replication in the areas of SCI neuroprotection and axonal regeneration. In this new program, entitled *Facilities of Research Excellence in Spinal Cord Injury (FORE-SCI)*, a list of potential studies to be replicated will be determined by a scientific research group, with collaborations from external advisors and NIH. This program will allow the most exciting and promising studies to be replicated by an independent laboratory, with the help of the principal investigator responsible for the originally published study. The study and findings will be published in peer-reviewed journals, as well as scientific society publications and NIH bulletins. Such a program should help accelerate the translation of important preclinical data to the treatment of human SCI.

PUBLICATION OF STUDIES

It is essential that experimental findings be published in peer-reviewed journals. This strategy not only allows scientists in the field of SCI to read about the experiment, but also allows for the critical assessment of the data and primary conclusions. Too often, studies are discovered when investigators visit other laboratories, or they are heard about second-hand from investigators in the field. This type of data communication is not satisfactory, because under these circumstances, critical factors such as study design, quantitative endpoints, and statistical analyses are generally not available for review.

It is also important to publish data from replication studies. Whether the study conclusions are positive or negative, the peer review process will allow other scientists to review data and arrive at their own conclusions. Hopefully, studies that are replicated by an independent laboratory and show benefit can be considered by the scientific community to be worthy of consideration for clinical application.

SAFETY ISSUES

Another critically important factor regarding moving a therapy to the clinic is the issue of patient safety. Safety issues should be considered at every step of the testing phase, including animal modeling, potential toxicities in rodents, and in nonhuman primates, if necessary. Such considerations may make separate preclinical benefit and safety studies necessary. Safety issues are particularly important in transplantation studies, where cell implantation into and around the injury site is considered. Because reparative approaches will almost certainly include invasive techniques, it is essential that the first treatments be delivered to a region of the spinal cord where any collateral damage from surgery will have limited or no adverse effects on the patient. For this reason, various research groups consider functionally complete lesions at the lower part of the thoracic cord to be the most favorable groups of patients to treat initially [23]. This is in contrast to the opinion that patients with cervical lesions would be expected to benefit the most from even minor degrees of regeneration.

The ASNTR has published guidelines that require consideration before a therapy is attempted in patients [24]. These include toxicity considerations, the possibility of biological contamination, and systemic effects, all of which should be reasonably balanced with possible benefit. These safety studies should be conducted in the best available model of the disorder.

CLINICAL STUDIES

A shortcoming of some clinical studies is that the design of the clinical trial failed to consider the limitations of the preclinical work. For example, factors such as dosing requirements and restricted therapeutic windows may have been ignored when the trial was designed. To illustrate, if experimental data indicate that a specific dosing response is critical for a neuroprotective agent to show efficacy, it is important that this dose be

mimicked in the clinical trial. Another factor that may significantly impact clinical studies is the therapeutic window for neuroprotection. Whether a drug can be administered to a patient in a predetermined window of opportunity presents an important prerequisite for successful treatments.

In terms of transplantation and restorative strategies, an understudied area of investigation is chronic SCI. Thus, critical questions require discussion, including animal modeling and the clarification of when transplantation strategies would be most effective in terms of acute versus chronic injury states. Obviously, there is a need in preclinical investigations to attempt transplantation strategies not only in the acute and subacute, but also the in chronic injury setting. To this end, experimental animals will have to be injured and allowed to survive for extended periods of time to mimic patient studies where reparative strategies are initiated years after injury [34,35]. At this time, few studies target the chronic injury state; this is one important area where new information is required.

Combination treatments targeting both neuroprotective and restorative processes after SCI are currently being tested in many laboratories. Researchers are discovering that a single treatment protocol alone may not be enough to protect a neuron from death or induce long-track regeneration and subsequent return of function. Thus, more complex strategies using two or more treatments are being evaluated [14,36]. To move these findings to the clinic, FDA regulations may require that each individual treatment be tested alone. Such a requirement would mean that researchers will need to pay attention to how the different agents interact, as well as make multiple comparisons between individual treatment groups.

CONCLUSIONS

As we begin to move into a new area of research discovery, in which experimental findings need to be translated into therapies that have realistic clinical applications, the scientific community as a whole must discuss a number of options. Questions regarding relevant animal modeling in both large and small animals require continued discussion. The ability of an independent group to replicate published findings appears to be a necessary condition for the consideration of new therapies for clinical investigation. Also, experimental findings should be robust and compelling to the scientific community, in terms of improvement in both structural and functional outcomes. We must continue to discuss what clinically relevant outcome measures are available to assess, both experimentally and clinically. Obviously, our major endpoint is to improve patient function and quality of life, and without appropriate assessment tools, it may be difficult to bridge the experimental and clinical research areas. As always, safety is of utmost importance when new therapeutic strategies are investigated. Surgical interventions, as well as the potential toxicity of compounds or cells injected into the human cord, are important considerations as we move forward. Finally, it is critical that findings are published in peer-reviewed journals so that scientists interested in the field can rigorously determine the risk-benefit ratio. Each of these goals must be achieved so that continued hope can be provided to those individuals living with paralysis following SCI.

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