Evidence from a Nonhuman Primate Model of Traumatic Spinal Cord Injury in Cynomolgus Macaques (Macaca fasicularis) to Evaluate for the Efficacy of a Combination Pharmacological Treatment

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Nonhuman primate model of tsci to evaluate efficacy of combination treatments

This article presents the results of experiments involving Macaca fasicularis monkeys, where the experimental lesions were created with a balloon catheter that was inserted into the epidural space. Prior to creation of the lesion, an EMG recording device is inserted; this facilitates measurement of tail movement and muscle activity before and after TSCI. This model is unique in that the impairment is limited to the tail; the subjects do not experience limb weakness, bladder impairment and/or bowel dysfunction. Four of the 6 subjects received a combination treatment of Thyrotropin Releasing Hormone (TRH), selenium, and vitamin E after the experimental TSCI. The subjects tolerated the implantation of the recording device and did not experience adverse effects due medications administered. The electromyographic data was transformed into a metric of volitional tail moment (“Q”). This metric appears to be valid measure of impairment to a measure of impairment and recovery. The histopathological assessment demonstrated widespread axon loss at the site of injury and areas cephalic and caudal. There was histopathological evidence of continuing inflammation, with macrophage activation. No treatment effect was recognized, based on the electromyographic data.

Abbreviations and Acronyms:
Quantitative Metric of Volitional Control - Q
Thyrotropin Releasing Hormone - TRH
Traumatic spinal cord injury – TSCI
Introduction

Traumatic spinal cord injury (TSCI) is a devastating clinical condition. The constellation of impairments includes limb weakness, dysesthesias as well as bowel, bladder and sexual dysfunction. Medical and rehabilitation interventions have improved quality of life, and long-term survival. Nevertheless, most people treated for TSCI continue to experience residual clinical impairments.

Relevance of Animal Models

Animal models are important in biomedical research. In the field of TSCI, a variety of species have been utilized, including mice, rats, cats, dogs, and rabbits as well as non human primates (NHP). In these models, the experimental spinal cord injury has been created by variety of methods. Some methods require surgical exposure of the spinal cord, with the lesion being effectuated with a sharp instrument or alternatively a device that provides static or dynamic compression. In other cases, the dura remains intact, and compression is applied with a circumferential clip. These approaches are complimentary. However, there are some pathophysiological and clinical features of TSCI that are similar to human beings, cats, rats, dogs and rabbits and NHP; however, there are also some substantial differences. For example, cats and dogs with a complete anatomical transection may be able to walk, this phenomenon is not observed in NHP or human beings. In this context, there is a significant role for NHP models in advancing the understanding of TSCI as well as identifying candidate treatments.

Focus of this article

This article presents the results of experiments involving NHP, where the experimental lesions were created with a balloon catheter that was inserted into the epidural space. Prior to creation of the lesion, an EMG recording device is inserted; this facilitates measurement of tail movement and muscle activity before and after TSCI. This model is unique in that the impairment is limited to the tail; the subjects do not experience limb weakness, bladder impairment and/or bowel dysfunction. Four of the 6 subjects received a combination treatment of Thyrotropin Releasing Hormone (TRH), selenium, and vitamin E after the experimental TSCI.

There are 2 key items in this article that are novel. First, this paper articulates the method by which the EMG data from the tail musculature can be utilized to formulate a quantitative measure of impairment and recovery from TSCI. This measure has been termed value “Q”. Quantitative measures of impairment and recovery in animal models compliment the subjective measures that focus of observation of limb function and/or movement.

Second, this article reports on the histopathological abnormalities associated with TSCI at the 90 day time frame post injury. This data is particularly valuable, as there are very few human anatomical specimens available at this time point. This reflects the epidemiology of human TSCI. Most people with SCI survive for years or decades. If, however, a patient dies, it is usually within the first few days after injury, or many years post injury.
Relevance of EMG data as a measure of impairment and recovery

EMG is a method by which muscle activity is electronically measured. The data can be obtained from electrodes placed on the surface of the skin or inserted directly into the muscle groups. In these experiments, the EMG data is obtained via implanted wire electrodes in the musculature of the tail. The EMG data was obtained before and after the creation of the experimental TSCI.

Conceptually, the magnitude of EMG signal is related to the amount of muscle activation in the muscle groups. For any individual muscle, there are a fixed number of motor units, which are the key functional units. A motor unit is defined as the motor fibers controlled by one motor neuron. These motor neurons are located in the spinal cord, and are controlled by neural networks in the spinal cord and brain. In TSCI, limb impairment, in part, is attributed to impaired functioning of the motor units.

In these experiments, the number of peaks were obtained from each subject as a feature to develop a “Q” score. Data collection encompassed the time periods before and after experimental spinal cord injury. Conceptually, this permits an assessment of impairment and recovery from TSCI, and provides an opportunity to evaluate the effect of potential treatments.

Rationale for experimental treatments

In these experiments, the candidate treatment administered was a combination of Thyrotropin Releasing Hormone (TRH), selenium, and vitamin E. In part, the choice of using these three agents was strategic as all three agents are currently available for human use. TRH is a tri-peptide produced in the hypothalamus. Selenium and Vitamin E are antioxidants. There is compelling evidence in animals that TRH, selenium and vitamin E modulate recovery in TSCI. As well, there is preliminary evidence in human TSCI to support the safety and efficacy of TRH. Specifically, TRH was administered to 20 patients with acute TSCI; the preliminary results were positive. Both Selenium and Vitamin E have been studied in clinical trials in other disease states and are well tolerated. There are, however, no animal or human studies to demonstrate the safety and efficacy of the combination of all three agents together.

Materials and Methods

Subjects enrolled

The subjects were six male healthy adult macaque male cynomolgus macaques (Macaca fascicularis) monkeys. These monkeys were free of Macacine herpesvirus 1, Simian T-Lymphotropic virus-1, Type D Simian retrovirus, and Simian Immunodeficiency virus at the time of the study. The subjects weighted between 6 to 13 kg. The subjects were not subject to previous scientific experiments or procedures. The first 4 subjects received combination treatment. The final 2 subjects did not receive treatment.

Housing and Husbandry of Subjects
Prior to the first surgical procedure, the subjects were socially housed, and resided in metal cages with another monkey. Immediately after the first surgical procedures, the subjects were housed in individual cages, in a room with other Macaca fasicularis. The subjects were fed twice per day a standard monkey diet (ZuPreem Primate Diet manufactured by Premium Nutritional Products or Teklad 2050 20% Protein Diet, manufactured by Envigo). The lights in the housing area were turned off for twelve hours per day. All subjects received an enrichment program.

Anesthesia and postsurgical pain management

All procedures were completed under general anesthesia and with aseptic technique.

Induction was achieved with ketamine (10-20 mg/kg IM) and atropine (0.03-.006 mg/kg IM). The hair in the area of the incisions was shaved. The skin was cleansed with alternating applications of surgical scrub and alcohol. An intravenous catheter was inserted, and normal saline or lactated ringers solution was administered at a rate of at a rate of 5-10 ml/hr. Subject were endotracheally intubated and mechanically ventilated. Anesthesia was maintained with inhaled isoflurane at 1-2 percent MAC. Continuous ECG and oxygen monitoring devices were used. The heart rate, blood pressure, temperature, and respiratory rate was closely monitored by the veterinary staff. The surgical sites were appropriately draped.

To manage pain related to the surgical procedures, Buprenorphine (0.005-0.03 mg/kg, IM) every 8-12 h or alternatively with Buprenorphine SR (0.06-0.2 mg/kg, SC) daily was administered. Additional analgesia was achieved with meloxicam 0.1-0.2 PO or SC daily. The initial dose of analgesic medications was administered in the pre-operative period. The margins of the surgical incisions were infiltrated with an equal mixture of lidocaine and marcaine.

Initial surgical procedure to facilitate collection of electromyographic data

On the lower back of each subject, a midline incision was placed superior to the proximal tail and a small pocket was dissected between the underling muscle and subcutaneous fat. This was followed by a small incision will be made on the left and right side of the tail to expose flexor cauda longus and brevis muscles; these muscles are an agonist-antagonist pair. A small telemetry device (Data Sciences, Minneapolis, USA) was surgically inserted into the pocket in low back. The telemetry device is attached via a set of wire electrodes, which were implanted into the left and right flexor cauda longus and brevis. The exposed areas of the electrodes were 10 mm; the distance between the active and reference electrodes was 5 mm. Figure 1 conceptually illustrates this experimental approach.

The tail of Macaca fasicularis can be considered the 5th limb, which is involved in the performance of functional tasks and balance. The tail has well developed sensory and motor areas. EMG data was collected via radiofrequency link to a computer for 30 d to determine baseline tail movements. Data collection occurred Monday to Friday (excluding holidays) for approximately 1 hour. The EMG responses were mathematically transformed into a quantitative metric for volitional control of tail movement; we have termed this metric “Q”. The method by which Q was calculated is described later in this paper. and
Subsequent surgical procedure to create experimental spinal cord injury

Thirty days post implantation of the transmitter, the subjects underwent a second surgery. The anesthesia and postsurgical pain management plan was administered as articulated earlier in this section.

A small laminotomy was performed at the fifth lumbar level. An epidural balloon-catheter was inserted and advanced approximately 10 cm cranial to the level of the lower thoracic spinal cord. The balloon was rapidly inflated, and remained inflated for a period of one minute. Conceptually, this corresponds to human SCI, to the extent that there is a rapid transfer of energy (i.e., initial balloon inflation); this is followed by residual displacement of tissues such as disk material, bone fragments, and/or hematoma (i.e., continued balloon inflation for 60 s). The balloon was then deflated. The catheter was removed and the surgical incision was closed.

Figure 2 is an x-ray highlighting the location of the experimental lesion in the low thoracic vertebral level. Figure 3 is a computed tomography image demonstrating how the lesion is created and the displacement of the thoracic spinal cord by the epidural catheter.

After the lesion was created, the subject remained under anesthesia for one hour. This is the typical time frame between human injury and availability of emergency medical treatment.

After one hour, four subjects (treatment) received an intravenous bolus of TRH at a dose of 0.2 mg/kg; this was followed by a continuous intravenous infusion of 0.2 mg/kg per hour for 1 hour. Selenium 60 micrograms and vitamin E 80 IU were administered orally once a day starting one day postsurgery and continuing for a period of 90 days. The first dose was on the first day post-surgery. Two subjects (nontreatment group) received an infusion of normal saline at the one hour time point. These control subjects did not receive any selenium or vitamin E. All the subjects experienced a spinal cord lesion.

In 5 subjects (three with treatment, and two without treatment), EMG data was recorded from day 0 (day of creation of the selective lesion) until day 90. In one subject, EMG data was obtained for 120 d post TSCI.

Monitoring of subjects

The subjects were closely monitored by the investigators and veterinary staff. The frequency of assessments was at least twice per day, and more frequent in the immediate postsurgical periods. The subjects were closely monitored for clinical indicators of illness such as limb weakness, vomiting, diarrhea, jaundice, bleeding as well as anorexia. Weight was obtained at the time of a surgical procedure as well as prior to euthanasia. Weights were also obtained at the time of routine health maintenance. The subjects were monitored clinically for wasting and emaciation, as a clinical indicator of weight loss.

Euthanasia and postmortem examination
At day 90, five of the subjects were humanely euthanized, with sedation with Ketamine (10-20 mg/kg intramuscularly) followed by administration of sodium pentobarbital (greater than 50 mg/kg). One subject (no treatment), was subsequently humanely euthanized 120 d post lesion. The post mortem examination was completed immediately after euthanasia. A gross and microscopic examination was completed, including as assessment of the brain, spinal cord, heart, liver, spleen, kidneys, and bladder. Venous blood was obtained immediately prior to euthanasia. Cerebral spinal fluid was also obtained.

The research on the first 2 subjects were completed at the New England Primate Research Center. The animals were cared for in accordance with the National Research Council’s Guide for the Care and Use of Laboratory Animals (8th edition, 2011) and the standards of the Harvard Medical School Standing Committee on Animals. Research on the last four subjects was completed at the Wisconsin National Primate Research Center and approved by the Institutional Animal Use Committee at the University of Wisconsin at Madison. The subjects were under the close supervision of the veterinary staff and were monitored for any adverse effects.

**Histopathologic assessment**

For histopathology, blocks were dissected from the epicenter of the lesion and sites caudal and cephalad. These blocks were fixed in 10% neutral buffered formalin for 7 d, embedded in paraffin and cut at 5 µm. Standard H&E staining was done on sections from all blocks. In addition, sample sections were stained with luxol fast blue (LFB) to highlight myelin changes.

Standard immunoperoxidase IHC for ionized calcium binding adapter molecule one (Iba1), a macrophage and microglia-specific marker, was also performed. Sections of brain and spinal cord were deparaffinized, rehydrated, and blocked with 3% hydrogen peroxide in PBS. Iba1 pretreatment involved microwaving for 20 min in 0.01 citrate buffer, followed by 20 min of cooling. Following pretreatment, an avidin-biotin block (Invitrogen Corporation, Frederick, MD, USA) and a Dako Protein block (10 min; Carpineria, CA, USA) were conducted on all sections. A wash of tris-buffered saline (TBS) followed each step.

**Analysis of EMG Data**

Conceptually, the overriding goal is to convert the raw unprocessed longitudinal EMG data into a metric volitional tail movement (i.e., “Q”). The collection and analysis of this EMG data of this nature is novel. Specifically, this data set contains EMG data in a longitudinal manner from the tail musculature. Furthermore, the muscles are an agonist-antagonist pair. The availability of prelesion and postlesion data is particularly valuable. In this context, there is no prescriptive or established analysis paradigm.

The statistical power in these experiments in enhanced by the collection of prelesion and postlesion EMG data. This permits the normalization of the postlesion data for each subject, and theoretically, decreases statistical variances. In addition, the prelesion and postlesion data was collected on a daily basis. Each recording date serves as a unique data point. This facilitates inferences related to the trajectory of recovery. In total, the data set contains approximately 800
million distinct observations. As a consequence, this experimental strategy decreases the number of subjects required to ascertain a treatment effect.

Of note, the data from the first subject was used to formulate a preliminary analysis paradigm, which was previously published. This preliminary paper focused on analyzing the number of turns and area under the EMG waveform. The determination of Q in this paper is substantively different from the previously articulated analysis strategy. Of particular note, in this paper, Q is calculated based on the number of peaks in the EMG data, which is a different EMG parameter. As well, the EMG data was processed with signal rectification, thresholding, and smoothing, as methods to emphasize times of high activity from nearby motor units, an aspect not used previously studied. As well, the analysis is based on entirety of the study population of these experiments.

In calculating Q, the EMG data was processed based on the following fundamental assumptions:

1. The lesion caused a perturbation to the EMG signal that was collected from the tail musculature.

2. The wire electrodes will detect all electrical EMG activity from all the motor units “close” to the electrodes. As motor units contain motor fibers, the EMG activity also represents the EMG activity of all the motor fibers as well.

3. The wire-tissue interface matures over time from the initial implantation. Therefore, the EMG signal later in the recording period is less likely to be affected by artifact related to surgical implantation.

4. The number of peaks in the aggregate EMG signal can act as a surrogate of motor unit (MU) activation. From a clinical perspective, this translates into greater tail movement.

The following steps summarize the signal processing strategy:

1. Filtering: Wavelet denoising was performed to eliminate noise. The objective of filtering is to retain as much of the original signal while concurrently attenuating “noise”.

2. Rectification: After filtering, the signals were rectified to convert all the negative values to positive values. This resolves positive-negative signal cancellation.

3. Smoothing: A moving average is calculated for a continuous segment containing 10,000 data points. This information was used to mathematically “smooth” the rectified EMG signals and reduce the effect of artifacts.

4. Thresholding: The spikes with low amplitude were discarded. This is to ensure that data from the motor units close to the electrode were imputed in the calculation of Q.

As a result of the signal processing strategy, the total no. of peaks per daily recording session were calculated. This value was reduced by the average (arithmetic mean) number of peaks for
each prelesion day (i.e., after insertion of transmitter, but prior to spinal cord lesion). Finally, this number was divided by the standard deviation of the number of peaks in the pre-lesion period. This approach “normalized” raw data based on the relationship to activity in the pre-lesion period.

As such, for every day of EMG recording, a value of Q was calculated. The values of Q were calculated for muscles of the left and right side of the tail separately. To determine if there was a difference of tail movement before and after the lesion, as well as to ascertain the possibility of a treatment effect with combination therapy, a linear regression model was used.

The first effect tested with the model relates to the electromyographic consequences of the lesion. Stated more explicitly, did the lesion cause a change in Q? The second effect tested relates to whether combination treatment results in improved Q scores, when compared to the absence of treatment. In other words, was combination treatment effective?

Due to the longitudinal nature of the study, the time rates of each effect were also tested. If found not to be significant, these effects were subsequently removed from the model. Data from the right and left tail muscles were tested separately. The analyses were conducted using a SAS statistical program (SAS Institute Inc., SAS 9.1, Cary, NC, USA) and MATLAB (Mathworks, Natick, MA, USA)

Results

Animal welfare related to experimental protocol and administration of combination treatment

The subjects were closely monitored by the investigators, veterinarians, and animal research technicians. Subjects were closely monitored for indicators of illness including limb weakness, vomiting, diarrhea, jaundice, bleeding and anorexia. All six subjects tolerated the implantation of the telemetry device and did not explant the device. None of the subjects required pain medications beyond the immediate 72 h time postsurgical time frame. The subjects did not traumatize their tails or engage in behaviors consistent with pain. Consistent with the experimental goals, there was no evidence of limb weakness and/or bowel or bladder dysfunction. The subjects were monitored for weight loss and there were no abnormalities.

The infusion of TRH is potentially associated with cardiac arrhythmias. In this protocol, the medication was administered while the subject was under anesthesia and had continuous ECG monitoring. No arrhythmias were identified in the treatment or non-treatment subjects.

Blood samples were obtained immediately prior to euthanasia with post mortem examinations performed immediately thereafter. Gross and microscopic examinations included assessment of all major organs. The postmortem examination was focused on evaluation of the brain, spinal cord, and potential systemic complications related to TSCI. For example, subjects with spinal cord injury can experience autonomic dysreflexia, which could result in hypertension, which may result in stroke and cardiac hypertrophy. Alternatively, TSCI can result in detrusor sphincter dyssnergia, which can be associated with post mortem features hydronephrosis and bladder hypertrophy. None of the subjects demonstrated evidence of pathology beyond the spinal cord. Furthermore, laboratory indices, including complete blood count, liver function tests and prothrombin times, did not demonstrate pathology that
could be potentially attributed to toxicity of selenium and/or vitamin E. This provides further reassurance of the animal welfare issues. The pathological findings in the spinal cord are described in the following section.

**Histopathologic features**

On gross examination, no significant abnormalities were noted. Specifically, no abnormalities were noticed in the vertebra, dura or substance of the spinal cord. The site of laminectomy was healed with both osseous and periosteal regeneration.

Microscopic evaluation of fixed tissues specimens was similar in all the subjects. Multifocally, within the white matter funiculi, there are many dilated myelin sheaths up to 75 µm in diameter. The axons were either swollen (spheroids) or lost by Wallerian degeneration with replacement by phagocytic microglia (Wallerian degeneration). There are areas of vacuolization (spongiosis), and collections of microglial cells (glial nodules) in both the grey and white matter. These findings were noted at the epicenter of the lesion as well as cephalad and caudal to the lesion.

At the site of injury, neurons in grey matter were often surrounded by more than 4 glial cells (satellitosis). Many neurons were swollen with a loss of Nissl substance and cellular detail (chromatolysis). The Nuclei were often faded with dispersed chromatin (degeneration).

Some representative microscopic images are noted in Figures 4-8. The qualitative histopathologic features were similar in all six subjects. The descriptions of the histopathologic features represent the consensus of 3 veterinary pathologists.

**Electromyographic features**

Although data was collected on all six subjects, in one subject, data from one side was not stored. As such, the analysis of the EMG data represents the experience to 3 subjects who received combination treatment and two subjects who did not receive combination treatment.

Table 1 summarizes the Q values at key time points: i.e., day of implant, midpoint of prelesion phase, immediately post lesion, midpoint of post lesion phase and termination of experiment. Figure 12 represents a fitted linear regression model for the aggregate data of Q during the pre and post lesion phase. The value of Q for both the left and right side of the tail increased during the pre-lesion period. This is attributed to a maturation of the wire-muscle interface.

Immediately after lesion, the value of Q decreased on both the left side and right side. This decrease in Q is noted in both the treatment and non-treatment group. An F-tests suggests that the difference in Q scores before and after the spinal cord lesion are statistically significant; left side (p = 0.021) and right side (p = 0.01). This suggests that the lesion led to decreased EMG activity resulting in decreased tail movement. This supports the construct that Q can be used as a measure of impairment after experimental TSCI.
On the left side, the treatment group was associated with a trend towards higher Q-values, when compared to no treatment (p = 0.075). This result is of borderline statistical significance. This effect was not noted on the right side (p = 0.519). Fitted Q values are presented in Table 1 showing normalized values for each subject at critical times during the experiment. The value of Q increased with time in both the treatment and non-treatment group, although the absolute values on both sides were higher with treatment (see Figure 12). Overall, the EMG data is insufficient data to impute an effect of treatment.

Discussion

Relevance of spinal cord lesion created with balloon inserted via epidural Catheter

In these experiments, the spinal cord injury was created with a balloon placed in the epidural space. The initial inflation of the balloon corresponds to the initial transmission of energy in human TSCI. Maintaining the balloon inflated corresponds to pressure on the spinal cord from structures such as disk material, bone fragments, hematoma et. The lesion was created without compromising the dura. This is consistent with most human TSCI. Specifically, there is no tear or laceration of the dura, with no leakage of cerebral spinal fluid. As previously reported, lesions created by this method have the same histopathologic features of human spinal cord injury during the acute phase (i.e., one hour post injury).

There are very few histopathology studies of human TSCI at three-month post injury. This reflects the epidemiology of the disease. Most people with SCI survive for years or decades. If, however, a patient dies, it is usually within the first few days after injury, or many years post injury. As such, there are relatively few anatomical tissues from individuals who died within 3-mo of TSCI. In this context, the data from these NHP subjects is particularly unique.

The lesions created by this method also appear to have histopathological features similar to human TSCI during the subacute phase (i.e., 90 days post injury). Human TSCI, at this time point, is characterized by axonal loss, demyelination, and microglial activation. This is consistent with the results of these experiments in nonhuman primates. Conceptually, this model is most relevant to TSCI at the thoracic level, and less relevant to cervical TSCI.

From a histopathologic perspective, TSCI can be classified as concussions, contusions, lacerations and solid cord injuries. Concussions are typically associated with transient neurologic complaint with no identifiable pathological challenges. Lacerations are the result of penetrating injuries caused by a knife wound or bullet. Contusions are associated with violent non-penetrating injuries, with substantive disruption of spinal cord architecture. Solid core lesions are associated with the maintenance of spinal cord architecture. The lesions created in these experiments are most consistent with solid core lesions.

In human TSCI, more severe injuries are associated with an epicenter of substantive disruption. Characteristically, there is a perimeter of less injured spinal cord structures. This model may be particularly relevant to understanding these less injured structures that may be most amenable to pharmacologic treatment.
It is striking to note that the subjects had histopathological substantive widespread axonal loss; this is despite a relatively focused lesion in the thoracic spinal cord. Speculatively, pro-inflammatory chemical mediators could be generated at the epicenter of the lesion and travel to other parts of the spinal cord via the rich and redundant vascular supply.

Of note, despite the extensive histopathological findings, the subjects did not have any clinical impairments such as limb weakness, bowel impairment, or bladder dysfunction. This is the context of clear neurophysiological evidence of impairment in the tail musculature. This would suggest that there may be greater resilience and/or redundancy in the spinal cord that has been previously appreciated. Alternatively, the lack of clinical impairment, despite substantive histopathological abnormalities, may implicate the importance of residual displacement and/or compression of the spinal cord in contributing to clinical impairment (i.e., herniated disk material, disrupted architecture of the spinal column due to fracture-dislocation, etc.). It is possible that limb weakness could have been predicated by using a larger balloon and/or increasing the time that the balloon remained inflated. That approach, however, would be adverse to animal welfare, and may also preclude long term survival of the subjects.

Animal models of TSCI are more challenged to demonstrate efficacy as opposed to safety. Safety can be more readily ascertained by close surveillance of clinical abnormalities and detailed review of laboratory and post mortem findings. Inferences related to efficacy in humans require the identification of animal behaviors that may correspond to human function. In human TSCI, the primary goal of treatment is to improve limb control to performing functional tasks (i.e., walking, dressing, feeding, bathing, etc.). Functioning motor units, as evidenced by the EMG data, are essential to attaining greater independence in functional tasks. In this context, the EMG endpoint is a meaningful measure of impairment and recovery.

It should be noted that different levels of neurological recovery are required for different functional tasks. In human beings with TSCI, bearing weight in the lower limb and walking requires a lower level of recovery when compared to manipulating objects in the upper extremity.

**Using NHP models to test safety and efficacy**

NHP models can be appropriately utilized to evaluate the safety of a candidate treatment. In these experiments, combination treatment with TRH, selenium, and Vitamin E did not result in any adverse side effects. In addition, the post mortem examinations did not demonstrate any evidence of toxicity. As a general construct, demonstrating safety in a NHP model is reassuring, when proposing to administer a candidate treatment in human beings. In this context, combination treatment is probably safe to offer in human clinical trials.

In human clinical practice, central nervous system lesions may affect the dominant and nondominant limbs in an asymmetrical manner. There is evidence that NHP demonstrate limb preference. Based on clinical observation, the tail of this species also demonstrates this phenomena. As such, the physiological and functional consequences of a central nervous system lesion may have disparate effects on the dominant and nondominant sides of the body.
The statistical power in these experiments is enhanced by the collection of pre-lesion EMG data. This permits the normalization of the post-lesion data for each subject, and theoretically, decreases statistical variances. In addition, the pre-lesion and post-lesion data was collected on a daily basis. As such, each recording date serves as a unique data point, allowing for inferences related to the trajectory of recovery. As such, this experimental strategy decreases the number of subjects required to ascertain a treatment effect.

Based on the EMG data in these experiments, Q can be used to evaluate impairment after TSCI. Specifically, Q is decreased immediately after experimental TSCI and increases over time. However, there is insufficient evidence to impute a treatment effect of combination treatment. Moving forward, the results from these experiments have provided insights into statistical variances of Q, and will serve as a guide for future experiments.

**Dosing of TRH, selenium and vitamin E**

The rationale for the combination therapy in TSCI in general, and this particular combination specifically, is published elsewhere. One of the goals of these experiments was to ascertain a preliminary understanding of the safety and efficacy of the combination of these 3 agents. This has not been previously evaluated in animal models or human studies.

TRH was administered to human beings who experienced TSCI in a clinical trial at the dose of 0.2 mg/kg bolus followed by an infusion of 0.2 mg/kg/hour for a period of six hours. For this study, this weight-based dosing paradigm was also used. Subjects assigned to the treatment group received a bolus of 0.2 mg/kg with a continued infusion of 0.2 mg/kg/hour continued for 1 hour, while remaining under anesthesia. While the infusion could have been maintained for a longer period of time, this may be associated with an increased risk of adverse effects related to anesthesia.

Selenium and Vitamin E are antioxidants, which may also positively modulate the pathophysiology of TSCI. In selecting the dose in the initial experiments, the investigators considered the clinical dosing experience in human beings. In the context of the weight of the monkeys, the dose was reduced to 20 percent of a reasonable human dose. The SELECT trial administered 200 mg of selenium and 400 IU of vitamin E. However, the US Office of dietary supplements has stated that a selenium dose of 400 mg per day is safe. As a compromise, for this study, a target Selenium dose of 300 mg was chosen. As such, dividing these dose by 5 results in an administer dose of 60 mg of selenium and 80 IU of vitamin E.

**Using NHP model to test efficacy with other candidate treatments**

The histopathologic data showing the presence of macrophages and/or microglia suggests that the inflammatory process continues at the 90-d time point. Inflammation is present at the site of injury and both rostral and caudal to the site of injury. Speculatively, this continuing inflammatory cascade may be modulated by new biologic agents such as natalizumab, alemtuzumab, mitoxantrone, ocrelizumab; these agents are currently used to treat multiple sclerosis, where neuro-inflammation implicated.
Limitations and future research opportunities

As an academic construct, one single animal model cannot entirely recapitulate the human experience. Although the histopathological findings with this model are similar to human TSCI, this model is particularly relevant to injuries associated with solid core lesions. The spinal cord specimens were evaluated with qualitative techniques. Moving forward, additional inferences may be potentially ascertained with quantitative approaches; this would be an opportunity for further investigation.

There are a number of potential alternative approaches to analyzing the EMG data. The EMG analysis paradigm that was utilized in this paper focused on the number of peaks in the EMG continuous signal. An alternative approach is to evaluate the data with the assumption that greater EMG activity translates to greater recruitment of motor units. Operationally, the aggregate EMG signal could be “decomposed” into single and/or groups of motor units. Another approach is to consider the nature of the EMG signal at the point of change in movement of tail. This would involve evaluating the periods of co-contractions between the agonist-antagonist muscles; there is some evidence that co-contraction is a clinical phenomenon in human TSCI. There is also the opportunity to obtain video images of tail movement concurrently with the EMG data, and integrate this data element in the analysis paradigm. Despite the technical challenges, this is an area of further refinement and potential research.

Conclusion

The EMG and histopathologic data from this research advances our understanding of TSCI. Additional research is required. Hopefully, this will translate into novel treatments for people with spinal cord injury.

References


**Figure Legends**

**Figure 1.** The transmitter to collect EMG data is surgically implanted in the low back, and connected to recording wire electrodes in the flexor cauda longus and brevis of the tail. The EMG data is transmitted by radiofrequency link to a computer. Adapted and modified from Nesathurai S, Graham WA, Mansfield K, Magill D, Sehgal P, Westmoreland SV, Prusty S, Rosene DL, Sledge JB. Model of traumatic spinal cord injury in Macaca fascicularis: similarity of experimental lesions created by epidural catheter to human spinal cord injury. J Med Primatology. 2006 Dec;35(6):401-4.

Footnote: This illustration is a modification of a previous drawing. A new drawing can be commissioned, to meet the requirements of the journal.

**Figure 2.** Standard radiograph of catheter inserted into epidural space via laminotomy in cadaveric subject. The balloon is not inflated in this image.

**Figure 3.** Computer Tomography Image of Inflated Balloon in Epidural Space CT scan of thoracic spine, with catheter inserted in the epidural space. The balloon is inflated with air, which appears black in the spinal column (red arrow). Note that 60 percent of column is occupied by the balloon, and the spinal cord is displaced. This image is from a cadaveric subject.

**Figure 4A.** Photomicrograph of the spinal cord at the epicenter of the lesion. The grey and white parenchyma are disrupted by dilated myelin sheaths (spongiosis; arrows), which are widely present. Haematoxylin and eosin stain. These findings are noted caudal and cephalad to the lesion, in both treated and untreated subjects. Low magnification.

**Figure 4B.** Photomicrograph of the spinal cord (white matter funiculi) at epicenter of experimental injury. There are many dilated myelin sheaths (spongiosis; black arrows) some containing degenerating or swollen axons (red arrows). These findings are noted caudal and cephalad to the lesion, in both treated and untreated subjects. Haematoxylin and eosin stain. High magnification.

**Figure 5.** Photomicrograph of the spinal cord cranial to epicenter of the spinal cord lesion. There are dilated myelin sheaths (black arrows) and swollen axons (spheroids; red arrows). Luxol fast blue stain. High magnification.

**Figure 6A, 6B.** (A) Photomicrograph of the spinal cord at the epicenter of experimental Lesion. Iba1 There is Iba1 reactivity within the white matter adjacent to the gray matter with spongiosis of the parenchyma. (B) Increased magnification of region denoted by the box in A. Iba1 immunohistochemistry DAB staining, counter-stained with haematoxylin. These findings suggest that substantial inflammation continues at the time 90 day time frame. High magnification.

**Figure 7.** Photomicrograph of the spinal cord grey matter at the epicenter of experimental lesion. This figure shows Iba1 positive cells at the level of the lesion. These microglia show the hypertrophic phenotype typical of activated microglia. Iba1 immunohistochemistry DAB staining, counter-stained with haematoxylin. High magnification.
Figure 8. Photomicrograph of the spinal cord cephalad to epicenter of spinal cord lesion. Immunohistochemistry using Iba1 antibody highlights microglial cells. Microglial cells depicted with black arrows illustrate an activated phenotype having thickened processes and enlarged cell body. Iba1 immunohistochemistry DAB staining, counterstained with haematoxylin. High magnification.

Figure 9. The aggregate fitted Q-values, representing tail movement, are plotted over the 90 d for the left (top) and right (bottom) sides. Values are normalized to range between 0 to 1 with higher values indicating greater muscle activity. Of note, in both the left and right side, the values of Q are reduced immediately after the lesion. On the left side, the effect of the lesion in the treatment group (red) was attenuated, when compared to the non-treatment group. On both the left and right side, there was an improvement in Q score over a period of time.

Tables

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Table 1. The standardized Q scores for each subject increase between the time of insertion, and reach a plateau in the pre-lesion period. This reflects maturation of the wire muscle interface. The Q score decreases immediately post lesion, and gradually increases with time. The values of Q as related to time are nonlinear.
Standard radiograph of catheter inserted into epidural space via laminotomy in cadaveric subject. The balloon is not inflated in this image.

47x167mm (96 x 96 DPI)
Computer Tomography Image of Inflated Balloon in Epidural Space CT scan of thoracic spine, with catheter inserted in the epidural space. The balloon is inflated with air, which appears black in the spinal column (red arrow). Note that 60 percent of column is occupied by the balloon, and the spinal cord is displaced. This image is from a cadaveric subject.

161x101mm (96 x 96 DPI)
Photomicrograph of the spinal cord at the epicenter of the lesion. The grey and white parenchyma are disrupted by dilated myelin sheaths (spongiosis; arrows), which are widely present. Haematoxylin and eosin stain. These findings are noted caudal and cephalad to the lesion, in both treated and untreated subjects. Low magnification.

164x149mm (96 x 96 DPI)
Photomicrograph of the spinal cord (white matter funiculi) at epicenter of experimental injury. There are many dilated myelin sheaths (spongiosis; black arrows) some containing degenerating or swollen axons (red arrows). These findings are noted caudal and cephalad to the lesion, in both treated and untreated subjects. Haematoxylin and eosin stain. High magnification.

165x123mm (96 x 96 DPI)
Photomicrograph of the spinal cord cranial to epicenter of the spinal cord lesion. There are dilated myelin sheaths (black arrows) and swollen axons (spheroids; red arrows). Luxol fast blue stain. High magnification.

182x136mm (96 x 96 DPI)
Photomicrograph of the spinal cord at the epicenter of experimental Lesion. Iba1 There is Iba1 reactivity within the white matter adjacent to the gray matter with spongiosis of the parenchyma. B. Increased magnification of region denoted by the box in A. Iba1 immunohistochemistry DAB staining, counter-stained with haematoxylin. These findings suggest that substantial inflammation continues at the time 90 day time frame. High magnification

167x98mm (96 x 96 DPI)
Photomicrograph of the spinal cord grey matter at the epicenter of experimental lesion. This figure shows Iba1 positive cells at the level of the lesion. These microglia show the hypertrophic phenotype typical of activated microglia. Iba1 immunohistochemistry DAB staining, counter-stained with haematoxylin. High magnification

164x123mm (96 x 96 DPI)
Photomicrograph of the spinal cord cephalad to epicenter of spinal cord lesion. Immunohistochemistry using Iba1 antibody highlights microglial cells. Microglial cells depicted with black arrows illustrate an activated phenotype having thickened processes and enlarged cell body. Iba1 immunohistochemistry DAB staining, counter-stained with haematoxylin. High magnification.

165x123mm (96 x 96 DPI)
The aggregate fitted Q-values, representing tail movement, are plotted over the 90 days for the Left (top) and Right (bottom) sides. Values are normalized to range between 0 to 1 with higher values indicating greater muscle activity. Of note, in both the left and right side, the values of Q are reduced immediately after the lesion. On the left side, the effect of the lesion in the treatment group (red) was attenuated, when compared to the non-treatment group. On both the left and right side, there was an improvement in Q score over a period of time.