Injuries to the lowest portion of the spine and the spinal cord commonly result in paralysis and impairment of bladder, bowel, and sexual functions. These injuries are usually referred to as conus medullaris and cauda equina forms of spinal cord injuries. Presently, no treatments are available to reverse the neurological deficits that result from these injuries. In this project, we aim to reverse neurological deficits, including bladder function, in a rat model of spinal cord injury, which affects the lowermost portion of the spinal cord. This part of the spinal cord and the associated nerve roots are called the conus medullaris and cauda equina. In our experimental model, nerve roots carrying fibers that control muscle function and pelvic organs, such as the bladder and bowel, are injured at the surface of the spinal cord. This injury mimics many of the neurological deficits encountered in human cases. For treatment purposes, we transplant human derived embryonic stem cells, which have been prepared to acquire properties of motor neurons, into the lowermost portion of the rat spinal cord after injury and surgical repair of nerve roots carrying motor fibers. The studies will evaluate both acute and delayed transplantation of human embryonic stem cells, which have acquired properties of motor neurons.

During the second year of the studies, we have developed improved protocols to increase our ability to produce large number of motor neurons from human embryonic stem cells. We have also developed improved methods to detect motor neurons during the neuron production process by using fluorescent reporters inside of the cells. The latter development is of great help when sorting and preparing cells with desired properties for transplantation studies. In addition, we have refined our surgical methods to make it less invasive, using a one-sided injury model instead of lesions on both sides of the spinal cord in rats. Specifically, bladder dysfunction can be assessed after a one sided injury of nerve roots and be evaluated using a combination of bladder pressure recordings and electrical recordings referred to as electromyography (EMG) from muscles along the urethra. The revised procedure is well tolerated by the rats and is a suitable approach for studies of chronic injury and cell-based long-term treatments. A research manuscript describing this improved experimental method and refinement has been submitted to a scientific journal and reviewed, and the manuscript is currently undergoing our revisions before being considered for publication. The experimental refinement will greatly assist with our long-term studies on the effects of transplanted motor neurons derived from human embryonic stem cells. We have also begun experiments using our refined model and cells, which now can be produced in high numbers and be identifiable during both the cell culture steps and during the animal studies. Initial tissues have been harvested and are being processed for morphological analyses.
treatments. A research manuscript describing this improved experimental method and refinement has been published. The
experimental refinement will greatly assist with our long-term studies on the effects of transplanted motor neurons derived from
human embryonic stem cells. We have also performed transplantations of embryonic human stem cell derived motor neurons into
the rat spinal cord and demonstrated surgical feasibility as well as survival of large numbers of neurons in the rat spinal cord.
Some of the transplanted cells also demonstrate anatomical markers for motor neurons after transplantation.

Reporting Period: Year 3

During the reporting period, we have continued to demonstrate that human embryonic stem cell derived motor neurons and motor
euron progenitors can be produced in vitro. These motor neurons and motor neuron progenitors are transplanted into the rat spinal
cord after a lumbosacral ventral root avulsion injury and repair of injured roots in the form of surgical re-attachment of the roots to
the spinal cord surface. The lumbosacral ventral root avulsion injury mimics cauda equina and conus medullaris forms of spinal
cord injury, an underserved patient population with paralysis of the legs and loss of bladder and bowel function. In this clinically
relevant injury and repair model in rats, we have during the past several months demonstrated that transplanted human embryonic
stem cell-derived motor neurons and motor neuron progenitors are able to survive in the spinal cord of rats over extended periods
of time with large numbers of neurons being detectable in the spinal cord grey matter at 1, 2, and 10 weeks after the injury, surgical
root repair, and transplantation of the cells. The long term viability of transplanted cells suggests integration of the transplanted
cells in the host tissues. Some of the cells show expression of motor neuron markers, such as the transcription factor Hb9, as
demonstrated by immunohistochemistry and light microscopy. Additional studies have been performed during this reporting period
to address whether the transplanted cells may extend axons into the replanted lumbosacral ventral roots. Interestingly, many
human axons were detected in the replanted ventral roots using immunohistochemistry and light microscopy for the detection of
human processes. Additional immunohistochemistry demonstrated that these processes contained neurofilaments, which are
characteristic for axons. In control experiments, we showed that avulsed roots, which had not been replanted into the spinal cord,
did not exhibit any human axons. As expected, surgical reconnection of lesioned ventral roots to the spinal cord is needed in order
for the axons of the transplanted human embryonic stem cell derived motor neurons and motor neuron progenitors to be extended
into avulsed ventral roots. Furthermore, in a series of sham operated animals without ventral root lesions, human motor neurons and
motor neuron progenitors were also transplanted into the rat spinal cord. Interestingly, the transplanted human motor neurons and
motor neuron progenitors were here also able to extend axons into ventral roots, even though the ventral roots had never been
lesions. We conclude that transplanted human embryonic stem cell derived motor neurons are capable of extending axons into
both intact ventral roots and into ventral roots, which had been avulsed and surgically reattached to the spinal cord using a
replantation procedure. In functional studies, we have performed urodynamic studies and voiding behavioral studies in rats after
the transplantation of human embryonic stem cell derived motor neurons and motor neuron progenitors. These studies are still
ongoing with additional experiments being performed. However, preliminary studies suggest that the combination of acute repair of
avulsed ventral roots and cell transplantation results in a gradual improvement of voiding reflexes. Ongoing studies are addressing
the relative contribution that may be provided by the replantation of avulsed ventral roots and by the transplantation of human
motor neurons and motor neuron progenitors into the rat spinal cord.

Repair of Conus Medullaris/Cauda Equina Injury using Human ES Cell-Derived Motor Neurons

Grant Type: Early Translational II
Grant Number: TR2-01785-A
Project Objective: The PI is planning to determine whether acute or delayed transplantation of human ES cell-
derived motor and autonomic neurons and neuronal precursors into the rat lumbosacral spinal
cord can replace lost motoneurons and PPNs and reinnervate the lower urinary tract after a
lumbosacral ventral root avulsion injury and surgical root replantation. If successful, our studies
may result in a new treatment strategy to restore bladder function in subjects with CM/CE
injuries, a vastly underserved patient population.
Investigator:

Name: leif Havton
Institution: University of California, Irvine
Type: PI

Disease Focus: Neurological Disorders, Spinal Cord Injury

Human Stem Cell Use: Embryonic Stem Cell

Award Value: $1,527,011

Status: Closed

Application Title: Repair of Conus Medullaris/Cauda Equina Injury using Human ES Cell-Derived Motor Neurons
Public Abstract:
Injuries to the spinal cord commonly result from motor vehicle accidents, traumatic falls, diving, surfing, skiing, and snowboarding accidents, other forms of sports injuries, as well as from gunshot injuries in victims of violent crimes. Injuries to the anatomically lowest part of the spinal cord, the lumbosacral portion and its associated nerve roots commonly cause paralysis, loss of sensation, severe pain, as well as loss of bladder, bowel, and sexual function. Lumbosacral injuries represent approximately one-fifth of all traumatic lesions to the human spinal cord.

As a result of the direct injury to the lumbosacral portion of the spinal cord, there is degeneration and death of spinal cord nerve cells, which control muscles in the legs as well as bladder, bowel, and sexual function. No treatments are presently available in clinical practice to reverse the effects of these devastating injuries.

In order to reverse the loss of function after lumbosacral spinal cord injury, replacement of the lost nerve cells is required. Recent research studies have identified some properties that are shared by spinal cord neurons responsible for muscle and bladder control. Human embryonic stem cells can now be prepared in research laboratories to develop properties that are shared between nerve cells controlling muscle and bladder function. Such nerve cells are particularly at risk of degeneration and death as a result of injuries to the lumbosacral spinal cord. Human embryonic stem cells, which have undergone treatment to obtain properties of muscle and bladder controlling nerve cells, are now very attractive development candidates for new cell replacement therapies after lumbosacral spinal cord injuries. The proposed feasibility studies will study the properties of such cells in a clinically relevant rat model for lumbosacral spinal cord injuries.

In Specific Aim 1, we will determine whether ACUTE transplantation of human embryonic stem cells, which have been treated to develop properties of specific lumbosacral spinal cord neurons, may replace lost nerve cells and result in a return of bladder function in a rat model of lumbosacral spinal cord injury and repair.

In Specific Aim 2, we will determine whether DELAYED transplantation of human embryonic stem cells, which have been treated to develop properties of specific lumbosacral spinal cord neurons, may replace lost nerve cells and result in a return of bladder function in a rat model of lumbosacral spinal cord injury and repair.

A variety of functional studies will determine the effect of the cell transplantation on bladder function, walking, and pain. We will also use detailed anatomical studies to determine in microscopes whether the transplanted cells have grown processes to connect with pelvic target tissues, including the lower urinary tract. If successful, the proposed experiments may lead to a new treatment strategy for patients with lumbosacral spinal cord injuries.
Statement of Benefit to California:

There are presently about 250,000 patients living with neurological impairments from spinal cord injuries (SCIs) in the United States, and approximately 11,000 new cases present every year. SCIs typically result in paralysis, loss of sensation, pain as well as bladder, bowel, and sexual dysfunction. No successful treatments are available to reverse the neurological deficits that result from SCI. Common causes for SCIs include car and motorcycle accidents, skiing, diving, surfing, and snowboarding injuries, traumatic falls, sports injuries, and acts of violence. California medical centers encounter a large proportion of the overall cases in the U.S. because of our large population, extensive network of freeways, and an active lifestyle with recreational activities taking place both along the Californian coastline and in the mountains.

The proposed development candidate feasibility project will capitalize on recent progress in human stem cell science and surgical repair of conus medullaris/cauda equina (CM/CE) forms of SCI. Human embryonic stem cell-derived neurons and neuronal progenitors, which express the transcription factor Hb9, will be transplanted into the conus medullaris in attempts to replace lost motor and autonomic neurons after a lumbosacral ventral root avulsion injury in rats. Surgical replantation of avulsed lumbosacral ventral roots into the spinal cord will also be performed in this clinically relevant model for CM/CE injury and repair.

If successful, our development candidate may reinnervate muscles and pelvic organs, including the lower urinary tract after CM/CE forms of SCI. Return of functional bladder control represents one of the absolute top priorities among the spinal cord injured population (Anderson, J Neurotrauma. 2004; 21, 1371-83). Successful recovery of bladder function after SCI is expected to have very significant impact on the quality of life of spinal cord injured subjects and markedly reduce health care costs.

Recovery of bladder function in spinal cord injured subjects would markedly reduce or eliminate the need for intermittent bladder catheterizations and indwelling bladder catheters. The number of visits in physicians' offices and already over-crowded California emergency rooms for bladder infections and other complications would be markedly reduced, thereby significantly reducing health care costs for both patients and our state. Improved neurological function among the SCI population is also expected to reduce care giver needs, thereby further reducing health care costs. The increased independence that will result from improved bladder control and concomitant possible recovery of other neurological functions, for instance in transfers and locomotion, will promote return to and participation in the work force for many individuals with SCI. These effects are also expected to bring a very positive effect to the California economy and increased quality of life for those living with an SCI.