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RESEARCHERS FIND WAYS TO ENCOURAGE SPINAL CORD REGENERATION AFTER INJURY

Animal studies suggest new protocols for helping human spinal cord injury patients

CHICAGO — Animal research is suggesting new ways to aid recovery after spinal cord injury. New studies demonstrate that diet affects recovery rate and show how to make stem cell therapies safer for spinal injury patients. The findings were presented at Neuroscience 2009, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news on brain science and health.

In other animal studies, researchers identified molecules that encourage spinal cord regeneration and ways to block molecules that discourage it. The findings may help shape therapies for the more than one million people in North America who have spinal cord injuries.

Research released today shows that:

- A diet high in fat and low in carbohydrates speeds recovery in rats with spinal cord injuries. The study suggests that dietary content may affect spinal cord injury recovery rates in people (Wolfram Tetzlaff, MD, PhD, abstract 542.10, see attached summary).
- In animal studies, stem cell implants pre-screened for "unsafe" immature cells helped repair injured spinal cords without dangerous side effects, like tumor formation. The findings suggest best practices for human stem cell therapies (Masaya Nakamura, MD, PhD, abstract 642.14, see attached summary).

Other findings discussed at the meeting show that:

- Researchers are discovering how to encourage the spinal cord to regenerate and form functional connections after injury. Growth factors, enzymes, and molecular tools show promising results in animal models (Eric Frank, PhD, see attached speaker's summary).

"Some injuries harm nerve cells, but the brain often recovers from stress, damage, or disease," said press conference moderator Oswald Steward, PhD, of the University of California, Irvine, an expert on spinal cord injury and synaptic plasticity. "We are learning a great deal about how to encourage the recovery process and harness the plasticity of the nervous system to offer hope to spinal cord injury patients," Steward said.

This research was supported by national funding agencies, such as the National Institutes of Health, as well as private and philanthropic organizations.

Related Presentation:

Symposium: **Regeneration and Sprouting of Sensory Fibers in the Spinal Cord**
Tuesday, Oct. 20, 1:30–4 p.m., Room S406A

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Abstract 542.10 Summary

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Low-Carb Diet Speeds Recovery from Spinal Cord Injury

Wounded rats fed a diet high in fat and low in carbohydrates recovered better

A diet high in fat and low in carbohydrates, known as the "ketogenic" diet, quickens recovery in paralyzed rats after spinal cord injury, according to new research. The findings were presented at Neuroscience 2009, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health. More than 10,000 North Americans suffer a new spinal cord injury each year and more than one million people live with such damage.

Patients recovering from spinal cord injuries are typically given high-calorie solutions containing large amounts of sugar intravenously as they heal, even though this nutritional plan has never been validated. Previous studies have shown that fasting is beneficial after partial cervical spinal cord injury in rats, but this strategy is unpopular with patients and clinicians.

In this study, researchers investigated the ketogenic diet as a fasting alternative. As is the case with fasting, a lack of carbohydrates forces the body to use fat as fuel. To test the diet, rats were put on either a standard or ketogenic diet immediately after undergoing a cervical spinal cord contusion. The rats on the ketogenic diet recovered faster: after 14 weeks, 54 percent used their injured paws 15 times more frequently than the rats on a standard diet.

"Our results suggest that a ketogenic diet might be an appropriate initial treatment to improve outcomes in human spinal cord injuries," said Wolfram Tetzlaff, MD, PhD, at International Collaboration on Repair Discoveries, and the study's senior author. "Although there are still many unanswered questions and more research is needed, the early results from these animal experiments support the rationale for human trials."

A ketogenic diet is already used as a therapy for epilepsy. Furthermore, animal studies during the past decade have shown that this diet may also be helpful for neurodegenerative diseases such as brain injury, Alzheimer's and Parkinson's diseases, and amyotrophic lateral sclerosis.

Research was supported by the Christopher and Dana Reeve Foundation, the Craig H. Neilsen Foundation, and the Canadian Institutes of Health Research.

Scientific Presentation: Tuesday, Oct. 20, 9–10 a.m. South Hall A

542.10, Ketogenic diet initiated after SCI improves functional recovery in rats
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TECHNICAL ABSTRACT: The ketogenic diet (KD) has been well recognized as an effective non-pharmacological treatment for different neurological disorders including epilepsy, Alzheimer's disease, brain injury and Parkinson's disease. In this study, we investigated the preclinical use of a KD as a potential alternative therapy for acute spinal cord injury. KDs contain a high content of fat, very few carbohydrates and variable amount of proteins. Due to the minimal dietary sources of glucose in KDs, the energy supply of the brain primarily comes from ketone bodies produced in the liver via the metabolism of fats. Previously, we observed improved behavioral recovery after a unilateral crush of the cervical spinal cord in animals fed a KD with an 7:1 ratio of fat to carbohydrate plus protein. However, the 7:1 ratio is higher than the ratio used clinically, which ranges from 2:1 to 4:1 due to higher protein content. Therefore, in the present study, we tested the effectiveness of the more clinically relevant 3:1 KD and compared it to a standard control diet (SD) and the previously used 7:1 KD after a left unilateral contusion of the cervical spinal cord in adult male rats. Blood β-hydroxybutyrate levels of animals fed the 7:1 or 3:1 KD were similar between the groups, reaching maximum levels during the first week after spinal cord injury. Although, daily caloric intake was similar between the three groups, the 7:1 KD group showed attenuated weight gain compared to the 3:1 KD and the SD control group. Behaviorally, forelimb usage during vertical exploration was profoundly affected after cervical spinal cord contusion. The rats on the SD used the ipsilateral forelimb less than 3% while rearing. The animals fed a 3:1 KD however showed a four to five fold increase in ipsilateral forelimb use to around 13% ($p<0.0001$). The rats on the 7:1 KD displayed a trend towards a better recovery. Additional behavioral tests and histology are currently being analyzed. Taken together, these preclinical results demonstrate for the first time that a KD effectively promotes behavioral recovery even when initiated after spinal cord injury. We conclude that patients with acute SCI may benefit from optimized diets and that there is an urgent need for revision/validation of the nutritional guidelines for acute SCI.

Abstract 642.14 Summary

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Identifying Safe Stem Cells to Repair Spinal Cords

Pre-screened stem cells aided injured mice without tumor development

Adult stem cells tested for defects before being implanted in the injured spinal cords of mice helped the animals recover with no cancerous side effects, according to new research. In recent years, scientists found that some experimental stem cell therapies can cause cancerous tumors. Pre-screened cells could result in potentially life-saving treatments without such side effects. These new findings were presented at Neuroscience 2009, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

"We tried to identify induced pluripotent stem cells from adult tissue that would be safe when applied to cell therapy for central nervous system disorders," said Masaya Nakamura, MD, PhD, at Keio University School of Medicine, a co-author of the study. "These results suggest that properly pre-evaluated cells may be a promising source for future transplantation therapy."

Here, the authors investigated the possibility of making transplantation therapies safer and more efficient by examining different types of stem cells. They generated 36 induced pluripotent stem cell clones, which differed in their origins and other characteristics. They found that the cell's origin was a crucial indicator of whether the cells would result in tumors.

Results showed that immature (undifferentiated) stem cells are more likely to form tumors than mature ones. The transplantation of "safe" cells into mice with spinal cord injuries resulted in the formation of new neurons, while "unsafe" cells sped recovery for a short period but ultimately formed tumors.

"This study confirms that before human clinical trials go forward involving treatment of central nervous system disorders with induced pluripotent stem cells, pre-evaluating each cell clone carefully is essential," Nakamura said.

Research was supported by the project for realization of regenerative medicine by the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Scientific Presentation: Tuesday, Oct. 20, 1–2 p.m. South Hall A

642.14, Transplantation of human iPS cell-derived neurospheres into injured spinal cord of NOD-scid mice
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TECHNICAL ABSTRACT: Induced pluripotent stem (iPS) cells have the potential to resolve the ethical issues and immunological rejection associated with embryonic stem (ES) cells. Recently, we have reported the effectiveness of transplantation of mouse iPS cell-derived neurospheres (iPS-NS) for spinal cord injury (SCI) in mice. In the present study, we performed the transplantation of human iPS-NS into injured spinal cord of NOD-scid mice to determine their differentiation potential and safeness as a cell source of transplantation for SCI. While residual undifferentiated pluripotent cells positive for Tra-1-60 and Tra-1-81 were not detectable in human iPS-NS by flow-cytometric analysis, human iPS-NS mainly differentiated into neurons and less glial cells in vitro. Contusive spinal cord injury at Th10 level was induced in female NOD-scid mice using an IH impactor. Nine days after injury, the mice were randomized to receive human iPS-NS, human ES cell-derived NS (ES-NS) or vehicle control. Functional recovery was assessed by using BBB/ BMS scoring scale weekly for 8 weeks. Both human iPS and ES-NS grafted animals showed significantly better functional recovery compared to the vehicle control group. Grafted human iPS-NS survived and mainly differentiated into neurons and less glial cells within injured spinal cord. However, 7weeks after transplantation, large tumors were observed in 2 out of 23 human iPS-NS grafted mice. Most of tumor cells were positive for Nestin and Vimentin, and some cells were also positive for GFAP. There existed higher percentage of Ki67 positive cells in these tumor cells (approximately 5%), compared to non-tumorigenic human iPS-NS. Further investigation should be required for safety about transplantation of human iPS-NS for SCI.

Speaker's Summary

Chair: Eric Frank, PhD (617) 636-6739
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Symposium: **Regeneration and Sprouting of Sensory Fibers in the Spinal Cord**
Tuesday, Oct. 20, 1:30–4 p.m., Room S406A

This symposium will discuss recent progress in promoting the regeneration and sprouting of sensory axons in the adult, mammalian spinal cord after injuries to dorsal roots. Dr. Smith will describe how exogenous expression of NGF within the cord induces robust regeneration of nociceptive sensory axons throughout the entire dorsal horn, both to appropriate and inappropriate laminae. Regeneration to specific laminae can be achieved, however, using slightly overlapping expression gradients of NGF and semaphorin 3A, a chemorepulsive agent for these axons. Although both paradigms lead to near normal recovery of thermal nociception, the addition of semaphorin reduces the number of regenerating axons by 40%, indicating more effective synaptic connectivity. Dr. Frank finds that blockade of inhibition by intrathecal administration of Nogo receptor ligands and stimulation of axon growth by systemic neurotrophin treatment both promote anatomical and functional regeneration of sensory axons into the cord. In contrast to the pattern of regeneration promoted by Nogo blockade, neurotrophin treatment leads to regeneration of sensory axons to their appropriate target areas, suggesting that guidance cues capable of correctly targeting axons persist in the adult cord. Dr. McMahon will describe the effects of degrading chondroitin sulfate proteoglycans (CSPGs) on sprouting of intact sensory axons after lesion of adjacent dorsal roots. Treatment with chondroitinase ABC facilitates reorganization of primary afferent inputs in the dorsal horn seen electrophysiologically with the emergence of new receptive fields, behaviorally as improved sensory and sensorimotor performance, and functionally as activity-dependent phosphorylation of kinases in second-order neurons. Dr. Darian-Smith will discuss synaptic and other changes within the cord following cervical dorsal rhizotomy that enable significant recovery of hand function in primates. Synaptic changes occur that are unique to this type of spinal injury. Axonal sprouting of pre-existing neurons projecting into the cord also potentially enable spinal remodeling. Endogenous neurogenesis comprises an additional response mechanism whereby newly formed neurons may contribute to functional recovery by augmenting existing neural circuitry.