

Original Article

GEMINI: Initial behavioral results after full severance of the cervical spinal cord in mice

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Abstract

Background: The GEMINI spinal cord fusion protocol has been developed to achieve a successful cephalosomatic anastomosis. Here, we report the preliminary data on the use of a fusogen [polyethylene glycol (PEG)] after full cervical cord transection in mice to facilitate the fusion of both ends of a sharply transected spinal cord.**Methods:** Cervical laminectomy and a complete, visually confirmed cervical cord (C 5) transection was performed on female albino mice ($n = 16$). In Group 1 ($n = 8$), a fusogen, (PEG) was used to bridge the gap between the cut ends of the spinal cord. Group 2 received the same spinal cord transection but was treated with saline. Outcome was assessed daily using a standard scale (modified 22-point Basso-Beattie-Bresnahan scale) and filmed on camera.**Results:** The PEG group (group 1) showed partial restoration of motor function after 4 weeks of observation; group 2 (placebo) did not recover any useful motor activity.**Conclusion:** In this preliminary experiment, PEG, but not saline, promoted partial motor recovery in mice submitted to full cervical transection.**Key Words:** Cephalosomatic anastomosis, GEMINI, PEG, spinal cord fusionVideo is Available on:
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INTRODUCTION

To achieve a successful spinal cord fusion as required during cephalosomatic anastomosis (CSA), an effective technique to assure rapid reinnervation of the body across the divided segments of the cervical spinal cord is necessary.^[2] In the proposed spinal cord fusion protocol called GEMINI,^[3] reapposition of two sharply severed cords brings in contact the gray matter cores in which a cellular, sensorimotor highway courses from the brain throughout the cord; this cellular core is expected to re-establish contact by regrowth of the severed connections among propriospinal cells and by acute “fusion” of the neural membranes that were transected acutely. Concurrently, the integrity of a

certain number of axons coursing in the long tracts of the white matter of the spinal cord will be restored. Several lines of experimental data support this concept (reviewed in^[3,8]).

This fusion of transected axons, as demonstrated experimentally (reviewed in^[3,8]), is made possible by

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exploiting fusogens, such as polyethylene glycol (PEG) or chitosan, i.e., substances that restore the integrity of acutely transected nerve fibers, with maximum benefit observed when applied locally to the point of transection rather than when administered parenterally (reviewed in^[2,8]).

We recently reported the effects of PEG applied to the severed cervical cord with initial recovery of motor evoked potentials at 1 hour in rats.^[5] Here, we report on the recovery of motor function after full cervical section in mice at 4 weeks.

MATERIALS AND METHODS

The experiment was carried out in accordance with the Animal Ethics Committees guidelines and was approved by the Institutional Animal Care and Use Committee of Konkuk University (Seoul, South Korea).

Surgery

Female albino (ICR: Imprinting control region) mice (25~30 g, $n = 16$) were anesthetized using zoletil and xylazine (3:1 ratio, 1 ml/kg). Cervical laminectomy was performed, and the muscles overlying the vertebral column reflected exposing C4-6 laminae and spinous processes; the C5 spinous process was carefully removed. After gently raising the cervical cord with a hook that atraumatically coursed all around the cord, severance was performed with surgical sharp blades #11 in two passes to visually confirm transection. PEG (PEG MW 400, Sigma-Aldrich, USA) (Group 1, $n = 8$) or phosphate buffered saline (PBS) (Group 2, $n = 8$) were applied to the cut area.

The muscle and fascia were sutured and the skin closed. Dextrose 5% solution (20 ml/kg) was administered daily via intraperitoneal injection. After consciousness was regained, breathing and heart rate were maintained autonomously, and body temperature was maintained at a constant level (27–29°C) in an incubator. To prevent dehydration, normal saline solution was provided with

total parenteral nutrition (TPN, Chong Kun Dang, Korea) through the tail vein and abdominal cavity 4 times a day.

Functional assessment

The modified 22-point Basso-Beattie-Bresnahan (mBBB) Locomotor Rating Scale,^[7] a commonly used scale that evaluates the recovery of sensorimotor function after spinal cord injury in rats, was used to assess locomotor recovery in an open field; i.e., when rats are left to themselves to move and feed in an enclosed but spacious pen (0 = paralysis, 21 = normal; all scores above 5 signify some useful voluntary action). Two independent, but not blinded observers, were used to evaluate the spinal cord sensory motor function of the animals after cord transection. Before testing, the animals' bladders were expressed because spontaneous bladder contraction often accompanies hind-limb activity. The mice were placed in an open field and were observed for 5 min. Functional recovery was analyzed by measuring the mBBB score once every 3 days.

RESULTS

Functional recovery

After 24 hours, very slight movements of the forelimbs were observed in all 8 treated animals [Figure 1]. In the control group, at the 2-week endpoint, all 8 mice had died, with no meaningful functional recovery (mBBB of forelimbs and hindlimbs: 2 to 4). In the PEG treated group, 3 animals died within 2 weeks, whereas the remaining ($n = 5$) animals showed considerable physiological improvement with the provision of TPN and saline solution during the study time. Recovery of motor function was observed in forelimbs and hindlimbs (average mBBB score: 8 and 5 points, respectively) by the end of week 4. In addition, on week 3, the intake of a small amount of food and defecation were possible, which suggests functional recovery of the nervous plexuses regulating the digestive system (versus thinning and blanching of the intestines in the controls).

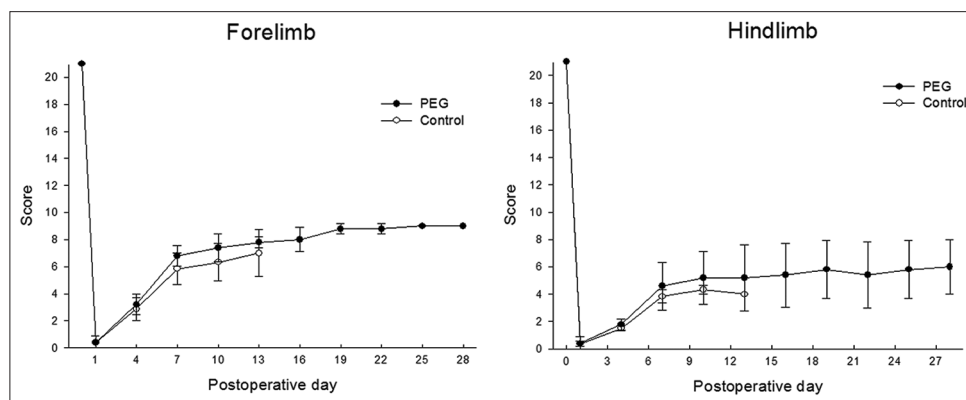


Figure 1: Modified Basso, Beattie, and Bresnahan (mBBB) scores for the forelimb and hindlimb in PEG-treated animals and controls

DISCUSSION

In this initial study, we tested one arm of the proposed GEMINI spinal cord fusion protocol, namely the use of fusogens applied topically to the sharply cervical severed spinal cord. We reported on the ultra-early results of PEG application,^[5] and showed that initial subnormal motor impulses could be seen crossing the point of fusion within 1 hour. Here, we extend those observations to include a longer follow-up (4 weeks). Mice reached a BBB score of 8 at 4 weeks [Supplementary video] without any kind of rehabilitative maneuver enacted or electrical stimulation, as expected in the full GEMINI protocol.^[3] Interestingly, this result is in line with a similar study in rodents where the completely severed dorsal cord was refused with a negative-pressure connector that gently kept the two stumps together and PEG circulated inside the connector.^[1] These rats reached a BBB score of 7 within 4 weeks, in agreement with our results.

In this behavioral study, histological analysis is not reported (manuscript in preparation), although preliminary analysis suggests that fibers regrew, local limited scarring notwithstanding. This is in line with the recent data that show that following spinal cord injury the astrocytic scar aids, rather than inhibits, axon regeneration, in rodents (the reader is referred to discussion in reference 5 for more details). This result^[5] is actually a mere reconfirmation of Freeman's original transection studies where fibers were seen crossing profusely the transection area (see in^[3]). Other studies also confirm that a scar is no impediment to resprouting in the spinal gray matter.^[4]

The death of rats after cervical spinal cord transection is not unexpected, and is likely due to cachexia following intestinal functional disturbance, as assessed at autopsy.

One deficiency of this work is that controls died before the end of the study. As Freeman observed, if given enough time, animals submitted to spinal transection will recover. In our study, controls also showed an initial recovery at 2 weeks, but of a lesser degree than

PEG-treated rats. No statistical analysis was conducted due to the small sample; also we could not compare rats at equal time-points. However, the data is already strong to confirm Freeman's observations, that a sharp section is not associated with permanent impairment.

In sum, in this preliminary set of data, we show that a severed cervical spinal cord does not lead to permanent motor impairment in mice if treated with GEMINI spinal cord fusogens, in agreement with other data at dorsal level.^[1,8] This experiment also supports the current efforts toward the first CSA in humans.

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Conflicts of interest

There are no conflicts of interest.

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