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Original Article

Chitosan/PEG-mediated spinal cord fusion after complete dorsal transection in rabbits – functional results at 30 days

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ABSTRACT

Background: The aim was to study functional recovery in experimental animals (rabbits) with transected spinal cords treated with a combination of photo-cross-linked chitosan in a homogeneous mixture with polyethylene glycol (PEG-chitosan).

Methods: 20 rabbits (n = 10 experimental and n = 10 controls) were submitted to complete spinal cord transection at T9. The experimental group received an intraoperative injection of PEG-chitosan. Neurological recovery was assessed using the modified Basso, Beattie, and Bresnahan scale.

Results: In the experimental group, partial recovery of movements, sensory function, and sphincter control were all observed by postoperative day 30. Paraplegia and anesthesia persisted in the control group; 4 controls died versus none in the test group.

Conclusion: PEG-chitosan is a candidate for neurological restoration after spinal paralysis.

Keywords: Chitosan, NeuroPEG, PEG, PEG-chitosan, Polyethylene glycol, Spinal cord injury, Spinal injury

INTRODUCTION

Since 2013, fusogens such as polyethylene glycol (PEG) have been tested in animal models of complete spinal cord transection with almost full recovery of ambulation.[1]

Here, we test a combination of PEG and chitosan[3,5,6] in rabbits submitted to complete dorsal cord transection and report behavioral results.

MATERIALS AND METHODS

The study protocol was approved by the Local Ethics Committee of the Stavropol State Medical University (March 12, 2020). This trial was carried out in accordance with the ethical standards

of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes.

We used chitosan with a molecular weight of 15 kDa (Merck, Germany), stabilized by photo-cross-linking, in a homogeneous mixture with PEG with a molecular weight of 600 kDa (PEG-600, Merck, Germany) at a concentration of 20 mg/mL (PEG-chitosan). The resulting mixture was sterilized and stored in sealed tubes at a temperature of 4°C.

Healthy male rabbits (n = 20) with a mean weight of 2.5 kg were anesthetized by injection of Zoletil (Virbac, France) and Xylazine (Alfasan int., Netherlands) (1:4) after premedication with atropine sulfate and droperidol. A standard T9 laminectomy was performed. The dura mater was dissected longitudinally over a distance of 5–7 mm along the midline. We inserted a flexible spatula into the subdural space between the anterior surface of the spinal cord and the posterior surface of the vertebral bodies. After that, we transversely transected the spinal cord using scalpel No. 11 so that the scalpel rested on the spatula. Hemostasis was obtained by irrigation with a cold (8°C) isotonic saline solution. In the experimental group (n = 10), we injected PEG-chitosan at the site of injury [Figure 1]. Saline was administered in the control group (n = 10). Closure was effected in standard fashion. Animals were placed in individual boxes for postoperative management. Analgesics, free access to water and feed, and an appropriate day-night cycle were all provided. The premises were air-conditioned, and the temperature was maintained constant.

Motor function was assessed on the modified Basso, Beattie, Bresnahan (mBBB) scale with a step test at a distance of 1 m and estimation of movements in joints. We tested the animals on a 1-m long horizontal ladder and a 2-cm ladder step. Sensory function was studied through needling the distal and proximal skin of the hind limbs, with quantification of reaction (0 = no reaction; 1 = vocalization (squeak, scream) in response to painful stimulation and/or attempt to remove the stimulus or escape from it (without limb movement); 2 = attempt to withdraw the limb; and 3 = active resistance to stimulus). We assessed diuresis, defecation, fullness of the bladder and intestine, and peristalsis.

Statistical analysis

The Kruskal–Wallis test was used for statistical evaluation. Calculations were made based on the clinical scores calculated on the mBBB scale for all postoperative days. Spearman's correlation coefficient was used to assess the correlation of results between groups. Comparisons between the two groups at multiple time points were analyzed with a repeated measures analysis of variance. Differences between groups were considered significant at $P < 0.05$.

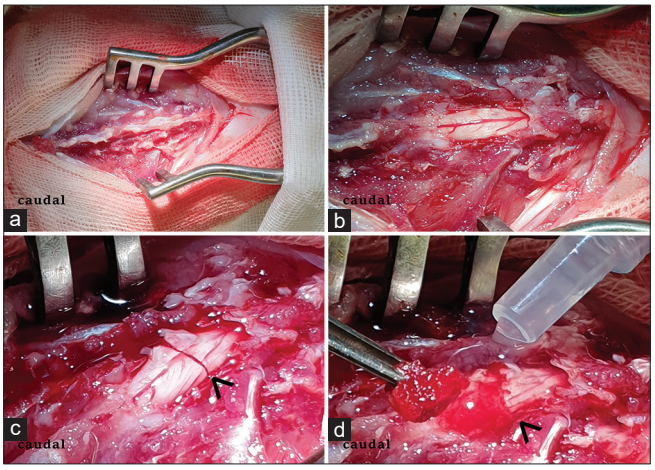


Figure 1: Operative steps. (a) Access to the thoracic vertebral arches. (b) Laminectomy and access to the spinal cord. (c) Transection of the spinal cord. (d) Intraoperative injection of polyethylene glycol-chitosan gel. The arrowhead indicates the site of the transection.

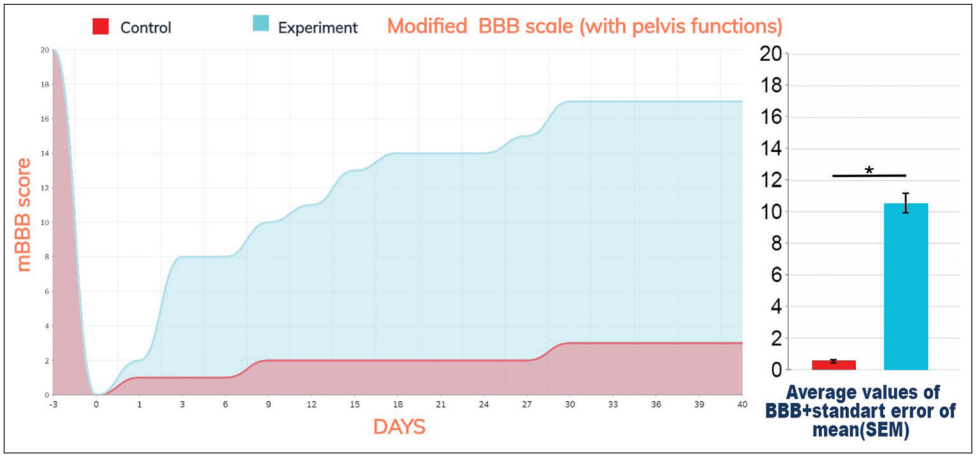


Figure 2: Locomotion, sensory, and pelvis functions as scored on the modified modified Basso Beattie Bresnahan (mBBB) scale. Data in graphs are shown as means ± SEM. The values significantly differed from the control at $P < 0.001$ ($P = 0.000251$ for comparison of the experimental and control groups). In particular, notice the rapid onset of recovery on POD 2 versus none in controls.

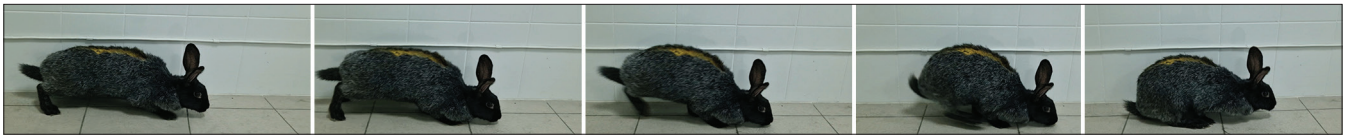
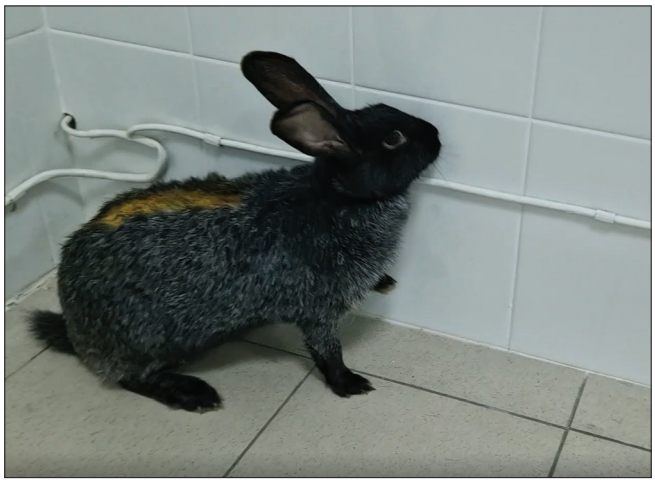


Figure 3: Treated rabbit (postoperative day 30). A short jump and an increase in the range of movements of the hind limbs are visible (video storyboard).



Video 1: Representative rabbit from the experimental group (POD 30). A short jump and an increase in the range of movements of the hind limbs are clearly visible.

RESULTS

Immediately after surgery, we observed lower paraplegia with below-level anesthesia and pelvic dysfunction (retention) in all animals. Controls showed no improvement in motor and sensory function throughout the experiment [Figure 2]. Pelvic dysfunction (retention) progressed after the 2nd day and required bladder drainage. Coprostasis regressed spontaneously, as a rule. One animal from the control group died after three days. An autopsy revealed a full bladder with dilated ureters, coprostasis, and partial ischemia of large and small intestines. Other three animals from the same group died after 5, 7, and 12 days with similar autopsy data.

Treated animals evinced the first signs of improvement after postoperative day (POD) 1. There were active movements of the hind legs in the horizontal plane (weak pulling of paws when moving) in one animal on POD 2. Five out of eight animals demonstrated strength in the hind limbs sufficient to push off and counter the experimenter's hand between POD 7 and 10. Weak movements and recovery of pain sensitivity were observed in all animals by day 14. Animals could move with support on the hind legs but could not jump. Pelvic dysfunction was transient by this time, and spontaneous emptying of the bladder and large intestine occurred after a 1-day delay as a rule. Mild lower paraparesis persisted by the 21st day, but the range of movements increased significantly. By the end of the experiment, slight paraparesis remained, but the range of movements allowed the animals to move freely, making small jumps [Figure 3 and Video 1].

Results were statistically highly significant (P = 0.000251, Spearman Coefficient: 0.91815).

DISCUSSION

In this study, we confirm that fusogens are effective in rabbits, similar to all other species tested so far.[1] We did not fully assess histological changes in our study. However, a preliminary study (unpublished data) shows – as per previous studies (fully reviewed in 2) – that fusogens neuroprotect the gray matter, which is considered the engine of recovery after such transections.[1]

In the current study, we did not tease apart the contribution of PEG and chitosan versus PEG versus chitosan alone, but this is clearly the objective of future studies. However, we notice that initial signs of recovery are seen after 24–48 h, in line with similar studies.[2] The findings are part of a series of experiments confirming the positive effect of fusogens on spinal cord injury in animal models.[3-7]

CONCLUSION

In sum, our results add to the growing literature on the potential of fusogens to restore the anatomophysiologic continuity of a transected spinal cord and – as such – can be leveraged for several approaches to the treatment of spinal cord injury.

Ethical approval

The study protocol was approved by the Local Ethics Committee of the Stavropol State Medical University (№87, March 12, 2020). This trial was carried out in accordance with the ethical standards of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes.

Declaration of patient consent

Patient's consent not required as there are no patients in this study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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