Regeneration of Hemisectioned Spinal Cords With and Without Supporting Materials

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Abstract

Nerve regeneration in hemisectioned spinal cords with and without supporting materials was examined in rats. Cell migration and newly formed capillaries surrounded by laminin-positive materials appeared at the lesioned site 7 days postoperatively. Reactive astrocytes increased at the lesioned site and extended into the spinal cord within 3 weeks. However, cell reactions decreased by 4 weeks and a cavity formed at the lesioned site in all animals. Regenerated axons were found only proximal to the lesioned site at 4 to 8 weeks. In the presence of supporting material (sciatic nerve containing proliferated Schwann cells or membrane filter), no cavity formed in the spinal cord and glial reactions were modest around the supporting material. Regenerated axons with growth cones were observed in the supporting material. However, no regenerated axons penetrated into the distal part of the spinal cord. Spinal cord axons can regenerate in the presence of supporting materials, but appropriate microenvironments, such as those found in the injured sciatic nerve, are required for spinal axons to traverse a lesion and penetrate into the spinal cord.

Key words: spinal cord injury, regeneration, peripheral nerve, spinal axon, graft

Introduction

Axonal regeneration and functional restoration in the central nervous system (CNS) were thought to be impossible in clinical practice. However, recent basic studies have demonstrated a significant regenerative capacity in the CNS. The cytoskeletal components of neurons are the same in the CNS as in the peripheral nervous system (PNS) and the processes of neurite formation and axonal regeneration are also similar as we previously observed in a culture system.^{5,8)} The primary difference appears to be the environment surrounding the neuron and neurite. Careful comparison of the regenerative processes, taking into account the environmental differences between the CNS and PNS, may suggest new approaches to enhancing CNS regeneration.

In the PNS, injured axons can regenerate and functional restoration is clinically possible. In the neurosurgical field, direct anastomosis with or without nerve grafts is commonly used to treat facial nerve injury after acoustic tumor surgery.¹⁵⁾ Hypoglossofacial or crossed faciofacial anastomosis is also effective for functional recovery of facial palsy.^{13,16)} The

regenerative capacity of peripheral nerve axons seems greater than that of central nerve axons, but the cellular and extracellular matrix microenvironments are important in the regeneration of axons in both the CNS and PNS.^{2,10,11)}

In the spinal cord, no axonal regeneration is expected clinically, and the outcome for patients with spinal cord injury is usually unsatisfactory. However, recent animal experiments using an antibody against myelin-associated neurite growth inhibitors have demonstrated a great regenerative capacity in spinal axons.¹⁸⁾ Regeneration of injured spinal axons has also been recognized in experiments with neuronal grafts and neurotrophic factors.^{3,17,22)} Functional restoration has been obtained by replacement of embryonic spinal cord segments in neonatal rats.⁹⁾ Recently, hind limb function in adult paraplegic rats has been restored partially by intercostal nerve grafts.¹⁾

The present study analyzed the regenerative processes of partially transected spinal cords with and without supporting materials in rats.

Materials and Methods

I. Animal model and implantation of supporting materials

Forty-nine adult male Wistar rats weighing 300–350 g were anesthetized by intraperitoneal injection of 4 mg/100 g sodium pentobarbital. A 10th thoracic laminectomy was performed with a diamond burr. The dura and arachnoid were opened and the left half of the spinal cord was transected with sharp scissors under the operating microscope. A piece of rubber sheet was placed on the spinal cord to prevent scar tissue invasion of mesenchymal tissue.

Implantation of supporting materials was performed after hemisection of the spinal cord in 29 animals. A membrane filter (cellulose acetate; Japan Millipore, Yonezawa, Yamagata) was implanted into the lesion immediately after transection ($n = 10$). Previously crushed sciatic nerves containing many proliferated Schwann cells ($n = 10$), or fibrin glue with ($n = 5$) or without ($n = 4$) nerve growth factor (NGF) (2.5S-NGF; Wako, Osaka) were implanted immediately and/or 3 to 4 weeks after injury.

II. Immunohistochemistry

The animals were sacrificed 3 to 330 days after operation by perfusion under deep anesthesia with a solution containing 1% glutaraldehyde and 4% formaldehyde in phosphate buffer. The thoracic spinal cords were dissected and embedded in paraffin. Proximal and distal portions of the lesions were studied in longitudinal and/or transverse sections. Immunohistochemical studies were performed by the peroxidase-antiperoxidase method using polyclonal antibodies against neurofilament (NF) (Dako, Carpinteria, Calif., U.S.A.), glial fibrillary acidic protein (GFAP) (Dako), and laminin (Cosmo Bio, Tokyo), and by the avidin and biotinylated peroxidase complex method using monoclonal antibodies to drebrin (developmentally regulated brain protein, provided by Prof. Shirao¹⁹). Drebrin is involved in brain development and plasticity.^{19,20}

Results

Round cells appeared at the lesioned site in the hemisectioned spinal cord 1 week postoperatively, and neovascularization was also observed at this time. Fibroblastic cells increased around the wound 2 weeks after injury and, 1 week later, extended into the spinal parenchyma. However, both cells and capillaries diminished and disappeared later, and a large cavity had formed at the lesioned site within 4 weeks (Fig. 1A). Cavity formation was observed in all animals sacrificed after 4 weeks. Some animals

recovered from paralysis; the anterior funiculus was preserved in these cases. However, most had permanent monoplegia of the hind limb. Laminin-positive substances appeared at 1 week only around vessels at the lesioned site (Fig. 1B). However, this substance later decreased and disappeared, along with the capillaries and migrating cells. Astrocytes positive for GFAP accumulated in the lesioned area and, within 3 weeks, extended into the spinal cord, especially the distal portion. Several months later, extensive glial scarring developed around the large cavity. Degenerating axons, observed as NF-positive retraction balls, appeared both proximal and distal to the lesioned site at 1 to 2 weeks. NF-positive regenerating axons were found proximal to the trauma site and growth cones were also observed after 4 to 8 weeks (Fig. 1C). However, no regenerating axons spanned the lesion. In control animals, some axons in the white matter, especially in the posterior funiculus and the gray matter, were slightly positive for drebrin. In lesioned animals, a strong positive reaction similar to that seen in the immunostaining for NF was obtained in regenerating axons (Fig. 1D). Many drebrin-positive axons were also found near the lesioned site in the contralateral posterior funiculus.

When a membrane filter was implanted as supporting material, no cavity was found at the lesioned site in any animals, and glial reactions were modest around the membrane filter even 11 months postoperatively. There were many axons with terminal enlargement in contact with the filter, and fine regenerating axons with growth cones were observed at up to 100 μ m depth in the membrane filter (Fig. 2A). Immunoreaction for NF was strongly positive in the terminal enlargement and growth cones of the regenerating axons. When sciatic nerves containing proliferated Schwann cells were used as supporting material, the grafts occupied the lesioned site and fused with the host spinal cords 2 weeks after transplantation (Fig. 2B). Some regenerated axons were found in the grafts 4 to 6 weeks after transplantation (Fig. 2C). Regenerating axons proximal to the lesioned site were dominant, and glial reactions were more modest in animals with grafts placed immediately after injury (Fig. 2D) than in animals with grafts placed 3 to 4 weeks after injury. However, the transplanted sciatic nerves degenerated gradually and disappeared thereafter. Fibrin glue with or without NGF implanted 3 to 4 weeks after injury disappeared and no residual materials were detected by 4 weeks after implantation. The wound was not sealed and the regenerating axons proximal to the lesioned site were not different from those in the lesioned sites without supporting material.

Fig. 1 Photomicrographs showing regeneration in the hemisectioned spinal cord. **A:** A large cavity was formed at the lesioned site within 4 weeks postoperatively. HE stain, $\times 200$. **B:** Laminin-positive reaction was found only around vessels at the lesioned site 1 week after injury. Immunostain for laminin, $\times 200$. **C:** Some regenerated neurofilament-positive axons were found proximal to the lesioned site 8 weeks postoperatively. Immunostain for neurofilament, $\times 400$. **D:** Drebrin-positive axons as well as neurofilament-positive axons were observed proximal to the lesioned site. Immunostain for drebrin, $\times 400$.

Discussion

This study found that initial cellular reactions and neovascularization at the lesioned site of the hemisectioned spinal cord disappeared and a large cavity formed. Glial reaction developed and extended distally along the spinal cord. Some regenerating axons with growth cones were observed proximal to the lesioned site but did not extend distally. Permanent monoplegia was the usual outcome. In contrast, our previous study of lesioned sciatic nerves found intense Schwann cell proliferation and laminin production at the lesioned site, which extended distally along the entire length of the

nerve.⁹⁾ Many regenerating axons extended distally and Schwann cells formed a myelin sheath around the axon. Finally, complete functional recovery was obtained. Immunohistochemical staining of regenerating axons for NF and drebrin did not reveal any significant differences between the sciatic nerve and the spinal cord, although some drebrin-positive axons were present in the spinal cord. The structural components of the CNS and PNS axons are the same but there may be differences in molecules such as receptors on the axonal membrane.

This study assessed the effects of supporting materials applied immediately and 3 to 4 weeks following injury on cavity and glial scar formation.

Fig. 2 Photomicrographs showing regeneration in the lesioned spinal cord with supporting materials. **A:** Regenerated neurofilament-positive axons extending into the membrane were found 11 months after injury. Immunostain for neurofilament, $\times 800$. **B:** A sciatic nerve graft containing proliferated Schwann cells fused with the host spinal cord 2 weeks after transplantation. HE stain, $\times 80$. **C:** Regenerated neurofilament-positive axons in the graft were found 6 weeks after transplantation. Immunostain for neurofilament, $\times 400$. **D:** Glial reactions were modest in the spinal cord with a graft transplanted immediately after injury. Immunostain for glial fibrillary acidic protein, $\times 80$.

Transplantation of embryonal spinal cords is known to achieve long-term survival⁷⁾ and connection formation between grafts,¹⁴⁾ but other materials were selected for possible clinical applications; an artificial matrix (membrane filter and fibrin glue), and autologous tissue (sciatic nerves containing proliferated Schwann cells). Both membrane filters and pretreated sciatic nerves inhibited glial scar formation and enhanced axonal regeneration. The inhibition of glial scar formation was significant when implantation was performed immediately after injury. Glial reactions were apparently suppressed by a block of mesenchymal cell in contact with the le-

sioned site. Since cavity formation was also reduced in these animals, the use of supporting materials may inhibit degenerative changes at the lesioned site. The enhancement of axonal regeneration by a membrane filter may be due solely to reduction of glial scar and cavity formation. Sciatic nerves containing proliferated Schwann cells enhanced regeneration of some spinal axons. Significant regeneration of optic nerve axons has been obtained by sciatic nerve transplantation.²¹⁾ The implantation of Schwann cells and peripheral nerve may therefore be suitable for some types of CNS axons. NGF has trophic effects for spinal axons,^{4,23)} so NGF in fibrin

glue was also implanted. However, no enhanced regeneration in spinal axons was seen. Other trophic factors such as neurotrophin-3 may be of more benefit for spinal axons.

Regenerated spinal axons were observed in the supporting materials. Many regenerated axons may be obtained using our previously described method if the supporting material is suitable for regeneration of spinal axons.¹²⁾ However, penetration and extension of the regenerated axons into the distal part and the entire spinal cord is more difficult. Our previous study of the lesioned sciatic nerve showed that proliferated Schwann cells along the whole length of the nerve enhanced and assisted the extension of regenerated axons. In contrast, the astroglial reactions along the entire length of the spinal cord may inhibit and disturb the regenerating axons. Myelin-associated neurite growth inhibitors are also present along the whole length of the spinal cord. Therefore, the microenvironments in the injured spinal cord may have a much greater influence on the regeneration of the spinal cord than the regenerative capacity of the spinal axons. We showed using intraocular double grafting methods that axons from the locus ceruleus can extend into the spinal cord and form synaptic contacts.⁶⁾ Embryonic spinal cords also seem to have the required environments for neurite extension and synapse formation. To achieve enhancement of spinal regeneration in adults, molecular approaches which can modify the microenvironments in the injured spinal cord should be considered. Comparative studies of molecular events in CNS and PNS regeneration may also be important.

In conclusion, supporting materials (sciatic nerves containing proliferated Schwann cells or membrane filter) implanted in the injured spinal cord prevented cavity formation and suppressed glial scar formation at the lesioned site. Regeneration of spinal axons was also enhanced by these materials. However, no regenerated axons penetrated into the distal part of the spinal cord. Appropriate microenvironments similar to those found in injured peripheral nerve may be required for spinal axons to traverse a lesion and extend into the spinal cord.

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Commentary

Spinal cord injury induces cavity formation around

the lesion site, which subsequently interferes with regeneration of the axons of the long tract of the spinal cord. In clinical cases of spinal cord injury, patients have not shown amelioration of symptoms due to cord injury such as paraplegia and anesthesia of the bilateral legs even after rehabilitation for long periods.

In the present article, the authors have studied pathological changes in the hemitranssected spinal cord in the rat. Using immunohistochemical methods, they have demonstrated that supporting materials such as cellulose membrane and sciatic nerves inhibited cavity formation after traumatic hemitranssection of the spinal cord and promoted regeneration of axons in the injured cord. Although the authors did not study functional changes in these animals, this new idea may be clinically applied in the patients with spinal cord injury in the near future.

Unfortunately the authors only briefly described the regeneration of some axons in the graft supported by cellulose membrane examined by immunohistochemistry. A count and statistical analysis of the number of cavities and axon fibers in the experimental animals would have been more useful.

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