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ScienceDirect
Journal of Clinical Neuroscience 15 (2008) 185–191
Journal of Clinical Neuroscience
www.elsevier.com/locate/jocn

Laboratory Study

# Chondrotinase ABC enhances axonal regeneration across nerve gaps

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Received 16 July 2006; accepted 17 December 2006

**Abstract**

We evaluated the effects of chondroitinase ABC on axonal regeneration across peripheral nerve gaps. We compared axonal regeneration after 15-mm tibial nerve resection and repair with a silicone tube filled with type I collagen gel (negative control group), with a silicone tube filled with type I collagen gel containing chondroitinase ABC at three different concentrations (2.5 units/mL, 5 units/mL, 10 units/mL) (chondroitinase ABC groups), and with an autologous nerve segment (nerve autograft group). Electrophysiological and histological assessments were carried out 12 weeks after surgery. In the electrophysiological study, compound muscle action potentials (CMAPs) and nerve conduction velocities (NCVs) were recorded in all groups except the negative control group. Although both CMAPs and NCVs were highest in the nerve autograft group, there were no significant differences among the three chondroitinase ABC groups in either parameter. Histological findings were consistent with electrophysiological results. Based on these findings, we conclude that topical injection of chondroitinase ABC can significantly increase the critical length of nerve gap repair by tubulization or artificial nerve placement.


*Keywords:* Chondroitinase ABC; CSPG; Peripheral nerve; Regeneration

## 1. Introduction

Chondroitin sulfate proteoglycans (CSPGs) are axonal growth inhibitors in the central nervous system (CNS). As in the CNS, CSPGs are abundant in peripheral nerve sheaths and the interstitium, and their expression is markedly upregulated following peripheral nerve injury,[1] and may impede axonal regeneration. In fact, Zuo successfully demonstrated that injection of chondroitinase ABC around the site of injury enhances axonal regeneration in a nerve transection/repair model.[1,2]

Chondroitinase ABC was first purified from *Proteus vulgaris* by Yamagata et al. in 1968.[3,4] This enzyme selectively degrades chondroitin sulfate and dermatan sulfate, and can be used for chemonucleolysis in patients with lumbar intervertebral disk herniation. Recent reports, including Zuo et al., have shown that surgical treatment of sites of injury with chondroitinase ABC is a promising method for enhancement of axonal regeneration in both the central and peripheral nervous systems.[1,2,6–9]

In contrast to the CNS, cells of the peripheral nervous system have a high degree of potential for regeneration following injury. In fact, meticulous nerve repair under operative microscopy usually leads to successful axonal regeneration across the suture line as long as the nerve stumps can be approximated without undue tension. In this sense, chondroitinase ABC treatment may be a useful though not indispensable adjunct in peripheral nerve repair on most occasions. This may not be true of more severe peripheral nerve injuries, in which significant nerve gaps are present. Although the distal stump of the transected nerve undergoing Wallerian degeneration secretes a number of diffusible neurotrophins such as nerve growth factor,

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doi:10.1016/j.jocn.2006.12.009

they can direct outgrowth of axons only over a distance of 5 mm or less.[10,11] Therefore, if a nerve gap of longer than 5 mm exists, use of an additional procedure to enhance axonal regeneration across the gap must be considered. At present, the gold standard for this is nerve autografting; however, this is necessarily associated with donor site morbidities. Another useful procedure is tubulization. In fact, most of the artificial nerves presently available are actually tubes made of various materials. As demonstrated by Lundborg,[12] axons can regenerate across a gap of up to 10 mm in rats within a silicone tube. A number of studies have been performed in attempts to increase the distance of repair with tubulization, such as administration of growth factors or axon growth-promoting extracellular matrix molecules.[13,14]

In the present study, we tested the hypothesis that digestion of axon growth-inhibitory molecules can significantly increase the gap that is repairable with tubulization. We used a 15-mm nerve gap rat model and evaluated the effects of chondroitinase ABC on axonal regeneration through a silicone tube. Both electrophysiological and histological assessments clearly demonstrated that administration of chondroitinase ABC into the silicone tube significantly enhanced axonal regeneration even at a very low concentration. Chondroitinase ABC thus appears to be a promising adjunct for expansion of the indications for use of tubulization or artificial nerves.

## 2. Materials and methods

Thirty-six Sprague-Dawley male rats 8 weeks old were used in this study. All surgical procedures were performed according to the NIH Guide for the care and use of laboratory animals, and the protocol was approved by the Nagoya University School of Medicine Animal Committee.

Rats were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg). After gluteal and posterior thigh incisions were made, the right tibial nerve was excised to create a 15-mm nerve gap. The proximal and distal stumps were bridged with a 15-mm long silicone tube (with an inner diameter of 1 mm and outer diameter of 2 mm; As-one Corporation, Osaka, Japan) using 8–0 nylon sutures. The lumen of the silicone tubes was filled with 0.8% type I collagen gel (Nitta gelatin, Osaka, Japan) containing chondroitinase ABC at four different concentrations. We selected concentrations of chondroitinase ABC according to Yick and Bradbury, who used this enzyme to enhance axonal regeneration in spinal cord injury models, as follows: 0 units/mL (negative control), 2.5 units/mL, 5.0 units/mL and 10 units/mL (chondroitinase ABC groups).[6,7] In addition to the silicone tube bridging model, we included a positive control group in which a 15-mm nerve gap was bridged by a nerve autograft instead of a collagen gel-containing silicone tube, and a sham group in which the rats were anaesthetized and a skin incision only was made. We thus divided the 36 rats into six groups of six rats each.

At 12 weeks after grafting, we performed electrophysiological and histopathological assessments. In the electrophysiological study, rats were anesthetized again and the tibial nerves were exposed bilaterally. Compound muscle action potentials (CMAPs) and nerve conduction velocities (NCVs) were measured with the Nicolet Viking IV (Nicolet, Madison, WI, USA). We compared the results obtained to those for the normal side (left). CMAPs and NCVs were expressed as percentages of values for the normal side. The tibial nerve was supramaximally stimulated at a point just rostral to the tube, and CMAPs were recorded from the ipsilateral gastrocnemius muscle. In order to calculate NCVs, the tibial nerve was stimulated both caudal and rostral to the bridging site, and the evoked waveform was added five times and averaged for precise measurement. Electrophysiological assessments were performed by an individual with expertise in operating the machine who was blind to grouping.

After electrophysiological assessment, the tibial nerves were harvested for histological assessment. The nerves were fixed in 10% buffered formalin and embedded in paraffin. Transverse sections were cut in the middle of the bridge. Immunohistochemical studies were performed using a monoclonal anti-neurofilament 200 antibody (Sigma-Aldrich, Tokyo, Japan) and a rabbit polyclonal anti-S-100 antibody (Neomarkers, Fremont, CA, USA). In brief, after deparaffinization and hydration, the sections were pre-incubated in 3% hydrogen peroxide and 10% normal rabbit serum to block non-specific reaction for 10 min, and then incubated with monoclonal anti-neurofilament 200 antibodies, which were diluted 1:100 in phosphate buffer at room temperature for 30 min. After washing with phosphate buffered saline, 10-min incubation with a secondary antibody at room temperature was performed. Sections were stained using a Histofine SAB-PO kit (Nichirei, Tokyo, Japan). For rabbit polyclonal anti-S-100 antibody, 10% normal goat serum was used instead of rabbit serum. Finally, sections were rinsed and incubated with the Liquid DAB Substrate-Chromogen System (Nichirei) for 10 min.

Electrophysiological data are expressed as the mean ± standard deviation. Results were analyzed using ANOVA without repeated measures, with the Student–Newman Keuls (SNK) test used for *post hoc* testing (Excel). Differences were considered significant at $p < 0.05$.

## 3. Results

CMAPs could be evoked in all groups but the control group (Fig. 1a). Percentage CMAP values were 15.7% in the 2.5 units/mL group, 15.3% in the 5.0 units/mL group, 29.5% in the 10.0 units/mL group, 49.6% in the nerve autograft group, and 99.8% in the sham group. The differences between the nerve autograft group and 2.5 units/mL and 5.0 units/mL groups were statistically significant. The differences between the nerve autograft group and all other groups were also statistically significant (Fig. 2a).