

NUS-01 Chondroitinase ABC promotes axonal regeneration of Clarke's neurons beyond the spinal cord injury scar

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Introduction: We have previously shown that enzymatic digestion of chondroitin sulfate proteoglycan (CSPG) at the injury scar promotes the axonal regeneration of Clarke's nucleus (CN) neurons into an peripheral nerve graft after spinal cord hemisection. The present study examined whether digestion of CSPG using chondroitinase ABC promoted the regeneration of CN neurons across the scar into the rostral spinal cord in neonatal and adult rats.

Method: Following hemisection of the spinal cord at T11, either vehicle or chondroitinase ABC was applied onto the lesion site. The postoperative survival periods were 2 and 4 weeks. Regenerating CN neurons were retrogradely labeled by Fluoro-Gold injection at spinal cord level C7.

Results: In the sham group, there was no regeneration of injured CN neurons in both neonatal and adult rats. Treatment with chondroitinase ABC in neonates resulted in 11.8% and 8.3% of the injured CN neurons regenerated into the rostral spinal cord, 2 and 4 weeks respectively. In adults, there were 9.4% and 12.3%, 2 and 4 weeks respectively, of the injured CN neurons regenerated their axons to the rostral spinal cord. After chondroitinase ABC treatment, the immunoreactivity for CSPG was dramatically decreased around the lesion site in both neonatal and adult animals.

Conclusion: Our results show that degradation of CSPG with chondroitinase ABC can promote the axonal regeneration in the spinal cord. These results further support the hypothesis that CSPG is inhibitory to the regeneration of neurons in the spinal cord after traumatic injury.

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NUS-02 Early release of mitochondrial cytochrome c and the subsequent activation of caspase-3 are involved in the apoptotic death of neonatal motoneurons after injury

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Introduction: We examined the mode of spinal motoneuron (MN) cell death after peripheral nerve injury in neonatal rats.

Method: Following root avulsion at C7 spinal segment in neonatal day 1 rats, either PBS or caspase inhibitors was applied onto the lesioned area. The postoperative period was 1, 2, 4, 6, 18, 24 and 48 hours. The time course of apoptosis was assessed by using TUNEL, nuclear staining and expression of cytochrome c and active caspase-3.

Results: Apoptotic features were first recognized in degenerating MNs by 6 hours after root avulsion. This was confirmed by both TUNEL and nuclear staining. Cytochrome c was released from the mitochondria into the cytosol as early as 1 hour following the lesion. Cytochrome c was localized preferentially in a diffuse pattern 1 hour after root avulsion whereas near the plasma membrane in normal motoneurons. By 6 hours after the injury, the active form of caspase-3 was first visualized by immunohistochemistry. Treatment with a caspase inhibitor Ac-DEVD-CHO could not completely block the activation of caspase-3 and the release of cytochrome c, whereas the administration of a pan caspase inhibitor Boc-D-FMK delayed the release of cytochrome c for more than 48 hours. These results implied that besides caspase-3, other caspase family members should be taken into account.

Conclusion: Because the release of cytochrome c and the activation of caspases play crucial roles in the apoptotic pathway, these results suggest that the mechanism of spinal motoneuron death after root avulsion in developing animals is apoptotic. Accordingly, inhibition of caspases may be a potentially important mean for rescuing immature MNs from injury.

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