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The relationship between changes in the expression of growth associated protein–43 and functional recovery of the injured inferior alveolar nerve following transection without repair in adult rats

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ABSTRACT

Objective: The objective of this study was to analyze the changes in the expression of growth associated protein–43 (GAP-43) in trigeminal ganglions (TGs) and in the distal stumps of transected inferior alveolar nerves (IANs), and to clarify the relationship between these changes and functional recovery of the transected IAN without repair using a rat IAN axotomy model.

Material and methods: Following transection, GAP-43 expression was measured at multiple time points. The functional recovery of the transected IAN was evaluated based on the compound muscle action potentials recorded from the digastric muscle.

Results: GAP-43 expression in TGs was significantly higher at 2, 7, 14, 28, and 56 days following IAN transection compared to that in samples from sham-operated rats ($p < 0.0005$, $p < 0.0005$, $p < 0.0005$, $p = 0.007$, and $p = 0.023$, respectively). GAP-43 expression in the distal stumps of transected IANs was significantly higher at 2, 7, 14, and 28 days following IAN transection compared to that in samples taken from sham rats ($p < 0.0005$, $p < 0.0005$, $p < 0.0005$, and $p = 0.009$, respectively). GAP-43 expression in the distal stumps of transected IANs returned nearly to sham levels by day 56 following IAN transection. On days 7, 14, 28, and 56 following transection, the amplitude of the compound muscle action potential gradually increased, the latency gradually decreased, and the duration gradually increased. The amplitude, latency, and duration of the compound muscle action potentials nearly returned to sham levels on post-transection day 56.

Conclusions: Time-dependent changes in the expression of GAP-43 in both TGs and distal stumps of transected IANs without repair are synchronously consistent with the regeneration and functional recovery of the transected IAN. The recovery of the amplitude, latency, and duration of the compound muscle action potentials indicates increased myelination and increased axon density of the regenerated nerve fibers.

1. Introduction

The inferior alveolar nerve (IAN) is part of the mandibular division of the trigeminal nerve and lies within a bony canal in the mandible in close proximity to the root tips of the mandibular molar teeth. The majority of the sensory neurons of the IAN are localized to the trigeminal ganglion (TG). The IAN can be damaged during the removal of the third molars or as a result of orthognathic surgery or mandibular fractures (Rustemeyer and Gregersen, 2012; Qinyong et al., 2014; Hillerup, 2008). Hillerup reported a significant spontaneous recovery in 66% of iatrogenic IAN injuries associated with the removal of the third molars (Hillerup, 2008). A review article showed 96%–98% sensory recovery of iatrogenic IAN injuries after bilateral sagittal split osteotomy (Antonarakis and Christou,

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2012). The incidence of post-traumatic IAN neurosensory dysfunction was reported to be 46% for mandibular fractures, and the incidence of persistent IAN sensory dysfunction following surgical treatment was reported to be 7.7% (Schultze-Mosgau et al., 1999).

Previous experimental studies have examined the regeneration of injured IANs and the reinnervation of the teeth after IAN ligation, crushing, and transection injury (Elcock et al., 2001a, 2001b; Long et al., 1998; Berger and Byers, 1983; Fristad et al., 1995; Iijima et al., 2003; Teramoto et al., 2013). The results of our previous study suggest that axon guidance cues such as Slit1/Robo2 signaling contribute to the regulation of the regeneration of the transected IAN without repair (Ceber et al., 2015). However, the microenvironmental mechanisms underlying the spontaneous regeneration and functional recovery of the transected IAN without repair are still largely unknown. Improving our understanding of the mechanisms regulating IAN regeneration is critical to the development of new therapeutic strategies aimed at accelerating the regeneration and functional recovery of injured peripheral nerves.

Peripheral nerve injury induces profound structural, biochemical, and physiological changes in both primary sensory neurons and peripheral nerves (Marchena et al., 1998; Bloechlinger et al., 2004; Teramoto et al., 2013). A key component of successful axonal regeneration in adult neurons is the induction of the neuron's regenerative competence (Chong et al., 1994; Teramoto et al., 2013). Growth associated protein–43 (GAP-43) expression is correlated with neuronal regenerative competence after axotomy (Yuan et al., 2009). The expression of GAP-43 in primary neurons increases as a result of neuronal damage, and this increase is terminated when the regeneration process is complete (Teramoto et al., 2013). Moreover, in the chronically denervated distal stump of the transected rat sciatic nerve, maximal GAP-43 expression was found in the absence of regenerating axons 4 weeks after transection (Curtis et al., 1992). However, longer-term GAP-43 expression has not been investigated.

Previous studies have shown a rapid increase in GAP-43 mRNA levels in cell bodies and proximal axon segments in motor neurons after axotomy (Palacios et al., 1994; Tetzlaff et al., 1991). A recent study has shown that IAN transection causes changes in GAP-43 expression in the TG (Teramoto et al., 2013). However, changes in the expression of GAP-43 in the distal stumps of transected, unrepaired peripheral nerves have not been evaluated. The regenerating proximal stumps of injured peripheral nerves elongate if they find favorable terrain. In the absence of a guiding structure such as a distal nerve stump, regenerating axons cannot elongate. Thus, determining whether there are changes in the expression of GAP-43 in the distal stumps of injured peripheral nerves may be important.

The objective of this study was to analyze changes in the expression patterns of GAP-43 both in TGs and in the distal stumps of transected IANs. We evaluated the relationship between these changes and the spontaneous functional recovery of the transected IAN without repair using a rat IAN transection model. The functional recovery of the transected IAN was evaluated using the compound muscle action potentials (CMAPs) recorded from the digastric muscle.

2. Material and methods

2.1. Animal surgery and tissue preparation

The experimental protocol used in this study was reviewed and approved by the local animal ethics committee in accordance with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals. A total of 74 male Sprague–Dawley rats weighing 250–300 g were used. For immunohistochemical analysis, 42 rats were anesthetized intraperitoneally with sodium pentobarbital (50 mg/kg). This surgical procedure was described in our previous study (Ceber et al., 2015). To expose each rat's right IAN, an extraoral horizontal incision approximately 10 mm in length was made in the skin covering the masseter muscle; the masseter muscle was partially separated, and the alveolar bone was exposed. The trajectory of the IAN was clearly identified through the transparency of the covering bone medial to the bony prominence in the body of the mandible. A small (approximately 0.5 × 0.5 cm) window in the outer part of the external cortex overlying the mandibular canal just above the angle of the mandible was removed slightly inferior to the prominence using a slowly rotating round dental bur under isotonic saline rinse until the nerve was visible and could be separated from its surrounding blood vessels. To preserve the integrity of the mandibular cortex medially and to prevent the complete penetration of both medial and lateral bony cortices, which are thin in the area distal to the bony prominence, the bone window was removed just mesial (anterior) to the bony prominence (Kassab et al., 2013). The inferior, superior, and medial circumference of the mandibular canal was preserved as a natural conduit for the regenerating axons. Using an operating microscope (Zeiss SV6/11; Carl Zeiss, Oberkochen, Germany), the exposed nerve was manipulated with fine microsurgical forceps at a magnification of ×16 to ×25. The IAN was carefully separated from the surrounding blood vessels and lifted from the mandibular canal, without any externally visible injury, by curved, angulated, thin glass rods. Then, the IAN was transected completely with a micro-scissors, without any further damage, using a meticulous and pressureless cutting technique. The cut ends of the transected IAN were immediately brought back into direct contact with each other and to their original position in the mandibular canal without any discernible gap between the cut ends and without any rotational error. The muscle and skin were then closed and sutured. The rats were allowed to recover and to survived for 2, 7, 14, 28, or 56 days (n = 7 for each period). Rats that did not undergo IAN transection were used as a sham treated group (n = 7); in this group, the skin was incised, the masseter separated, and the mandibular canal fenestrated. All rats were given the antibiotic penicillin G procaine (Phoenix Pharmaceutical, Inc., St. Joseph, MO) the day before, the day of, and the day after surgery (30,000 U/250 kg). After transection, rats were fed powdered food. Transected rats at 2, 7, 14, 28, and 56 days following transection and sham-treated rats at day 0 (n = 7 per group) were sacrificed. Rats were sacrificed using an overdose of sodium pentobarbital (>50 mg/kg, intraperitoneally). The TG and hemimandible of the transection site were quickly harvested and immediately flash-frozen in dry ice in preparation for immunohistochemical analysis. Hard tissues from the right sides of the harvested hemimandibles were decalcified for 3 weeks using 10% ethylenediaminetetraacetic acid (EDTA) in 0.1 M phosphate buffer. The right sides of both the TGs and decalcified hemimandibles were stored at −80 °C until sectioning.

For quantitative analysis of the CMAPs, unilateral axotomy of the right IAN was performed in 32 adult rats. CMAPs recorded from the digastric muscle were measured in sham-operated rats (n = 4) and on days 7, 14, 28, and 56 following the IAN transectionsq (n = 7 per time point).

2.2. Immunohistochemistry of the TG and hemimandible

The TG tissue that was collected for immunohistochemical study was frozen in dry ice and cut into 20-μm sections using a cryostat (Leica Instruments, Wetzlar, Germany). To ensure the examination of samples from the distal IAN stump, the hemimandible distal to the removed bone window was sectioned longitudinally,