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Nerve regeneration techniques respecting the special characteristics of the inferior alveolar nerve☆

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ABSTRACT

Purpose: The aim of this study was to examine the in situ regeneration of the inferior alveolar nerve (IAN) in its bony channel, using autologous tissue in combination with a recombinant human nerve growth factor (rhNGF).
Materials and methods: A total of 20 New Zealand rabbits were randomly divided into five groups. Following dissection of the IAN, the animals underwent reconstruction either with muscle tissue (groups 1 and 2) or with fat tissue (groups 3 and 4). In group 5 (control), the dissected nerve was resected and reconstructed by placement of the reversed autologous segment. After 2 and 4 weeks, 1 mL rhNGF was locally injected in groups 1 and 3. Nerve function was monitored by measuring the jaw-opening reflex using electromyography for a period of 24 weeks.
Results: Regeneration of the nerve was achieved in all groups, but preoperative threshold values were not achieved. Comparing the experimental groups to the control, there was a significant difference in favor of the autologous nerve reconstruction. Differences between the experimental groups remained statistically not significant.
Conclusion: Regeneration of the IAN with autologous tissue is possible, but without achieving preoperative thresholds. Additional injection of a growth factor seems to improve the speed of regeneration for fat and muscle grafts.

1. Introduction

Traumatic injuries of the inferior alveolar nerve (IAN) result mostly from extraction of wisdom teeth, apicoectomy, local anesthesia, implant surgery, maxillofacial trauma (Hillerup, 2007; Tay and Zuniga, 2007; Basa and Dilek, 2010; Jones, 2010), and malignancies.

Summing up the most frequently performed dental surgical operations (extraction of wisdom teeth, osteotomies, and cystectomy), 2.2% of cases have been reported to result in a sensory disturbance of the inferior alveolar nerve, according to a study with 1107 surgical interventions (Schultze-Mosgau and Reich, 1993). Solely considering the surgical removal of mandibular third molars, the incidence of injuries of the IAN has been reported to be 5%, with 0.3% of the cases resulting in permanent damage of the nerve (Valmaseda-Castellon et al., 2001). In this context, nerve damage during insertion of dental implants might occur more frequently, especially in elderly patients, due to atrophy of the lower jaw (Marquardt et al., 2007; Basa and Dilek, 2010).

Sequelae of nerve damage may be loss of pulp sensibility and sensory disturbances corresponding to the area supplied by IAN like hypesthesia, anesthesia, or paresthesia of the gingiva, lower lip,

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and the mental region causing massive restrictions in speaking, kissing, eating, drinking, and therefore a significantly lower quality of life (Abarca et al., 2006; Hood et al., 2009; Cheung et al., 2010; Pogrel et al., 2011). Moreover, neural dysfunction may cause chronic pain disorders (Poort et al., 2009; Zuniga, 2009).

If the injury of the nerve is diagnosed intraoperatively, coadaptation of both nerve ends should be performed. If the injury is not recognized immediately, differentiation between temporary or permanent damage of the IAN may be challenging if not impossible (Poort et al., 2009). Handling of such complications might be very difficult, especially regarding the fact of an increasing number of implants inserted by dentists not familiar with surgical nerve repair (Lambrecht et al., 2010). Treatment of neurotmesis depends on the length of the gap between both nerve segments. Hausamen et al. recommended a tension-free anastomosis with sutures to improve the healing process (Hausamen et al., 1974). Larger nerve defects are problematic: If the proximal end of the nerve does not reach the distal nerve stump, neuromas can develop (Wolford and Stevao, 2003).

If the proximal and distal nerve segments cannot be adapted closely, autologous nerve transplants, e.g., from the greater auricular or sural nerve are the gold standard (Jones, 2010). A clinical problem frequently results from different sizes and lengths of donor and receptor nerves and additional donor site morbidity (Gordon et al., 2003; Wolford and Stevao, 2003). Clinical treatment procedures for larger gaps usually do not yield a satisfying outcome (Hausamen et al., 2003).

Alternatively, alloplastic materials, e.g., silicone, polyester, collagen, or polyethylene, have been used to bridge the nerve defect in special cases. In addition, some of these conduits were filled with nerve growth factors and used as a delivery system (Scholz et al., 2010; Richner et al., 2014; Wang et al., 2014). The success rate of allografts appears to be compromised, probably due to foreign body reactions (Mackinnon et al., 1984; Pitta et al., 2001). The use of absorbable materials could be a another option (Meek et al., 2001). Their use is controversial because of the risk of disease transmission, e.g., Creutzfeld-Jacob (Ramasamy et al., 2003); however, satisfying results have been reported (Brooks et al., 2011). Further options for bridging nerve defects include the use of tendon, artery, muscle, vein, and fat grafts (Konofaos and Ver Halen, 2013). All of these options are used only in trials, and the clinical results are often unsatisfactory.

As mentioned before, growth factors are frequently used in nerve regeneration protocols. In particular, recombinant human nerve growth factor (rhNGF) is well known to promote nerve regeneration (Levi-Montalcini and Hamburger, 1951; Levi-Montalcini, 1976; Gundersen and Barrett, 1979, 1980; Campenot, 1982) and has been studied in preclinical animal studies using rabbits (Strauch et al., 2001; Zhang et al., 2002).

Many studies have examined nerve regeneration of peripheral nerves running free in body tissue (Iwakura et al., 2012; Ding et al., 2014; Giusti et al., 2014; Isaacs and Browne, 2014). In contrast to other studies, the IAN is a sensitive nerve surrounded by an envelope of bone. The nerve channel may work as a guide for the regenerating nerve, which might have a positive effect on healing. Studies using nanomaterials as guiding membranes confirm such assumptions (Saracino et al., 2012, 2013). In clinical trials using veins and muscle grafts for nerve reconstruction, a beneficial effect was observed if grafts were shielded against the surrounding tissue (Brunelli et al., 1993; Battiston et al., 2000a, 2000b).

The aim of this study was to establish an animal model for analyzing the healing of defects of the IAN with an objective method to monitor healing. Additionally, we sought to determine whether defect reconstruction with autologous tissue (muscle or fat tissue), with and without application of the growth factor rhNGF, could be compared to the gold standard (donor nerve), and we assessed IAN regeneration by electromyography (EMG).

2. Materials and methods

The study protocol was in accordance to the German animal welfare Act (Animal Experiment Permit: V312-72241.121-14 12-1/11). A total of 20 female New Zealand rabbits, 3 months old and with an average weight of 2.25 kg, were used for the study. The rabbits were divided into 5 groups ($n = 4$; Table 1) to test muscle and fat tissue for IAN repair, both also combined with NGF. The jaw opening reflex (JOR) of the rabbit can be used to test the function of the IAN (Renton et al., 2005; Fukuhara et al., 2011). The afferent branch of this brainstem reflex innervates the rabbit snout (IAN) and the efferent branch projects bilaterally to the digastric muscle (Mostafeezur et al., 2009). Using electrical stimulation, the JOR can be provoked and measured using EMG (Rupprecht et al., 2005). Reappearance of reflex response after nerve injury has been demonstrated to be a good indicator for axon regrowth and reorganization of sensibility of the snout.

2.1. Nerve monitoring of the JOR

The nerve function was monitored by the JOR before and after nerve dissection and 12, 16, 20, and 24 weeks postoperatively (Fig. 1). Two needle electrodes with a diameter of 300 μm were inserted near the anterior digastric muscle to record the motor response of the ipsilateral and contralateral digastric muscle, and two needle electrodes with a 300-μm tip diameter were vertically inserted submucosally in the first premolar region near the mental nerve foramen. The IAN was stimulated ipsi- and contralaterally before ipsilateral nerve dissection, to ensure correct function of the digastric muscle. The threshold of the reflex response of both digastric muscles was recorded. Rectangular electric impulses of 500 ms at a frequency of 0.1 Hz were set. Measuring range was 0.0 mA–10.5 mA and intensity was gradually increased in steps of 0.5 mA. The lowest stimulus intensity evoking the JOR was noticed and defined as threshold. If there was no reflex response, measurement was stopped and noticed as missing response. Fig. 2 depicts a typical reflex response of the digastric muscle. Electromyographical monitoring with conduction over the digastric muscle was recorded from 3 ms before to 100 ms after electrical stimulation, with a sampling rate of 20 Hz and high filtering from 100 to 1000 Hz. For the recordings, a diagnostic EMG machine (EMG Xltek® Neuromax, Canada) was used.

2.2. Surgery

Surgery and all electrophysiological measurements were performed under general anesthesia using Rompun® (Bayer, Leverkusen, Germany; Xylazin 2% 0.2 mL/kg body weight) and Ketavet®, (Upjohn, Heppenheim, Germany; Ketamin, 10%, 0.5 mL/kg body weight). Depth of anesthesia was controlled by blink reflex and depth of breathing.

All rabbits were shaved in the submandibular area, and disinfection was performed using Kodan Tinktur forte® (Schülke & Mayr, Norderstedt, Germany). For better control of contamination, an extraoral access to the right mandible was prepared. After submandibular incision with a scalpel (Nr. 15, Martin, Tuttlingen, Germany), preparation of the mandible from the angle of the lower jaw to the mental nerve was performed unilaterally. The vestibular bone was removed by Piezosurgery® (Mectron, Cologne, Germany). Following dissection (2 cm) of the nerve (neurotmesis) with a scalpel (Nr. 15, Martin, Tuttlingen, Germany), the animals either underwent reconstruction by muscle tissue from the musculus