Neuroscience 241 (2013) 157–169

SCIATIC NERVE REGENERATION IS NOT INHIBITED BY ANTI-NGF ANTIBODY TREATMENT IN THE ADULT RAT

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Abstract—Elevated nerve growth factor (NGF) is believed to play a role in many types of pain. An NGF-blocking antibody (muMab 911) has been shown to reduce pain and hyperalgesia in pain models, suggesting a novel therapeutic approach for pain management. Since NGF also plays important roles in peripheral nervous system development and sensory nerve outgrowth, we asked whether anti-NGF antibodies would adversely impact peripheral nerve regeneration. Adult rats underwent a unilateral sciatic nerve crush to transect axons and were subcutaneously dosed weekly for 8 weeks with muMab 911 or vehicle beginning 1 day prior to injury. Plasma levels of muMab 911 were assessed from blood samples and foot print analysis was used to assess functional recovery. At 8-weeks post-nerve injury, sciatic nerves were prepared for light and electron microscopy. In a separate group, Fluro-Gold was injected subcutaneously at the ankle prior to perfusion, and counts and sizes of retrogradely labeled and unlabeled dorsal root ganglion neurons were obtained. There was no difference in the time course of gait recovery in antibody-treated and vehicle-treated animals. The number of myelinated and nonmyelinated axons was the same in the muMab 911-treated crushed nerves and intact nerves, consistent with observed complete recovery. Treatment with muMab 911 did however result in a small decrease in average cell body size on both the intact and injured sides. These results indicate that muMab 911 did not impair functional recovery or nerve regeneration after nerve injury in adult rats. Published by Elsevier Ltd. on behalf of IBRO.

Key words: nerve growth factor, peripheral nerve regeneration, anti-NGF treatment.

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Abbreviations: ANOVA, analysis of variance; BLQ, below level of quantitation; DRG, dorsal root ganglion; FG, Fluoro-Gold; NGF, nerve growth factor; PK, pharmacokinetics; SFI, Sciatic Functional Index.

0306-4522/13 $36.00 Published by Elsevier Ltd. on behalf of IBRO.
http://dx.doi.org/10.1016/j.neuroscience.2013.03.024

INTRODUCTION

Increasing evidence over the past two decades has implicated nerve growth factor (NGF) and TrkA NGF receptors in the development of a broad array of persistent pain syndromes including chronic headache (Sarchielli et al., 2001), exercise-induced muscle soreness (Andersen et al., 2008), interstitial cystitis (Liu et al., 2009), pancreatic cancer (Zhu et al., 1999), and neuropathic pain (Radtke et al., 2010) (see reviews by McMahon (1996), Woolf (1996), Nicol and Vasko (2007), Watson et al. (2008)). Local injections of NGF have been shown to induce hyperalgesia in both humans (Dyck et al., 1997; Andersen et al., 2008; Rukwied et al., 2010; Deising et al., 2012) and rodents (Lewin et al., 1994; Ruiz et al., 2004), while blocking NGF or TrkA receptors with antibodies or fusion proteins decreases pain in rodent models (Woolf et al., 1994; Koewler et al., 2007; Guerios et al., 2008). Preliminary clinical trials on patients suffering from osteoarthritis (Lane et al., 2010; Nagashima et al., 2011) or interstitial cystitis have suggested that a humanized anti-NGF antibody, Tanezumab, can significantly reduce chronic pain (Evans et al., 2011).

Although treatments targeting NGF show promise for pain control, NGF also plays an important role in the development of the peripheral nervous system, raising concerns that anti-NGF therapies might impede recovery after peripheral nerve injury in adults. NGF acts as both a survival factor for embryonic (Levi-Montalcini and Angeletti, 1968; Ruit et al., 1992) and neonatal (Yip et al., 1984; Hulsebosch et al., 1987) dorsal root ganglion (DRG) neurons and as a guidance cue for embryonic DRG neurons (Letourneau, 1978; Gundersen and Barrett, 1979; Gallo et al., 1997; Paves and Saarma, 1997; Moore et al., 2006). In addition, NGF promotes neurite outgrowth from DRG and superior cervical ganglion neurons of all ages (Niwa et al., 2002). The timing and location of NGF and NGF receptor expression during nerve regeneration also suggest that NGF levels may be regulated by axon/Schwann cell contact and play an important role in axon guidance and/or myelination (Taniuchi et al., 1986; Heumann et al., 1987). Cosgaya et al. (2002) have demonstrated that the p75 receptor may be a positive modulator of myelination. Furthermore, the axon caliber of transected adult nerves can be increased by the direct application of NGF to the severed nerve stump (Gold et al., 1991) arguing that NGF continues to regulate the morphology of adult DRG neurons.

Several studies have reported that manipulation of NGF levels in adult animals affected the speed or efficiency of peripheral nerve regeneration or the survival of axotomized sensory neurons but the findings have been somewhat contradictory. Localized delivery of NGF was found to enhance early nerve regeneration in some studies (Rich et al., 1987; Savignat et al., 2008), but delayed regeneration in others (Young et al., 2001; Hirata et al., 2002). Consistent with their greater dependence on NGF during embryonic development (Ruit et al., 1992), regeneration of nociceptive axons may be more sensitive to NGF levels than other sensory neurons. Young et al. (2001) demonstrated that exogenous application of NGF to transected sciatic nerve stumps delayed recovery of noxious thermal withdrawal responses but did not affect gait recovery. Similar to the effects of NGF on regeneration, both localized over expression of NGF (Hu et al., 2010) and focal application of anti-NGF antibodies (Streppel et al., 2002) have been reported to reduce inappropriate collateral axon sprouting and improve targeting efficiency. More detailed studies investigating the effects of NGF and anti-NGF treatments on subtypes of DRG fibers suggested that collateral sprouting of nociceptive neurons was specifically influenced by NGF exposure (Diamond et al., 1992a,b). Diamond et al. (1992a) reported that collateral sprouting of nociceptive axons was inhibited by anti-NGF serum and stimulated by exogenous NGF although functional tests showed that Aα-mediated touch, Aδ mediated mechano-nociception and C fiber-mediated heat nociception were all unaffected by anti-NGF treatment (Diamond et al., 1992b). In general, systemic changes in NGF availability have been reported to produce little effect on adult sensory neurons, while localized infusions of NGF have been observed to influence DRG survival and/or nerve regeneration. Long term (5–6 months) exposure of uninjured adult rats to anti-NGF antibodies did not cause loss of DRG neurons (Gorin and Johnson, 1980), and systemic administration of NGF failed to increase survival of DRG neurons axotomized close to their cell bodies (Tandrup et al., 1999). However, localized infusions of NGF did reduce DRG neuronal loss when axons were transected farther from the cell body (Otto et al., 1987; Rich et al., 1987; Ljungberg et al., 1999), and intrathecal administration of NGF delayed peripheral nerve regeneration (Hirata et al., 2002). Taken together, these findings imply that changes in NGF levels or disruption of local NGF gradients could potentially impair peripheral nerve regeneration, but the role of NGF in peripheral nerve regeneration in the adult remains uncertain.

In this study we evaluated the effects of systemic anti-NGF monoclonal antibody (muMab 911) administration on functional recovery, axon regeneration (myelinated and nonmyelinated), and DRG neuronal populations in an adult rat sciatic nerve transection model. The results indicate that muMab 911 administered at a dose found to be therapeutic in rodent pain models (Halvorson et al., 2005; Shelton et al., 2005; Sabsovich et al., 2008) did not impair axonal regeneration or functional outcome, arguing against a functional effect of this degree of NGF inhibition in peripheral nerve regeneration.

EXPERIMENTAL PROCEDURES

All experiments were performed in accordance with National Institutes of Health guidelines for the care and use of laboratory animals. The Veterans Affairs Connecticut Healthcare System Institutional Animal Care and Use Committee approved all animal protocols. A total of 30 animals were used for three parallel experiments, 16 for behavioral analysis and quantification of axonal regeneration, six for muMab 911 blood level time course measurements, and eight for retrograde labeling of DRG neurons. Additionally blood levels of muMab 911 were determined at weekly intervals, just prior to dosing, in all rats. All analyses were performed by investigators who were naive with respect to treatment condition and who did not have access to the results of other analyses before all data were collected and analyzed. Morphometric analyses of nerves and DRG were also performed by different investigators who were not informed which nerve was crushed. After morphometric data for each animal were collected, animals in the two treatment groups were identified to investigators and data for each treatment group were then pooled and analyzed before the identity of the treatment groups and side of nerve crush were revealed.

Nerve crush

Adult female Sprague–Dawley rats (170–200 g) were anesthetized with 1–2% isoflurane gas and the left sciatic nerves were exposed using aseptic techniques and tightly crushed with #5 Dumont microforceps for 10 s in one direction and an additional 10 s at 180° rotation from the initial crush. This procedure leads to complete loss of function (Hildebrand et al., 1985) and transection of all axons at the crush site, but leaves the epineurium and perineurium intact. The crush site was standardized to the nerve region near the piriformis muscle tendon.

Drug injection and blood sampling

One day prior to nerve crush and each week thereafter, animals were weighed and injected with 10 mg/kg muMab 911 in PBS-Tween (1× PBS, 0.01% Tween 20, pH 7.4) or an equivalent volume of PBS Tween buffer subcutaneously in the fat pad near the shoulder blades. Individual animals were randomly assigned to control or experimental groups and co-housed in cages of three without respect to treatment group. The person who performed the injections and blood draws was not involved in behavioral or histological analysis so that all analyses could be blinded with respect to treatment condition. Immediately before each injection, a 150 μl sample of blood was taken via saphenous vein, processed for plasma and stored −70 °C for later analysis of muMab 911 concentrations. The terminal blood draw was obtained at the time of perfusion from the heart of the anesthetized animal. In addition to weekly blood draws from all lesioned animals, 150 μl blood samples were also taken from six nonlesioned satellite animals for composite full PK (pharmacokinetic) profiles at 1, 3, 6, 24 and ∼168 h following the first and last doses. In order to minimize stress, blood samples were drawn from all rats at the 168 h time point, but other blood draws were staggered with three animals sampled at 1, 6, and 168 h post dose and three animals sampled at 3, 24 and 168 h post dose.