Effects of Etanercept on axonal regeneration after peripheral nerve injury

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Introduction: The peripheral nervous system is remarkable in its ability to regenerate after injury because of a local permissive environment and activation of the neuronal intrinsic growth capacity. Local induction of inflammatory cytokines, which are produced by macrophages and Schwann cells, plays a crucial role in controlling Wallerian degeneration (WD) and nerve regeneration (1). Tumor necrosis factor-alpha (TNF) is one of the pro-inflammatory cytokines that is implicated in the initiation of WD and neuropathic pain (2). TNF inhibits axonal outgrowth in hippocampal neurons (3) and cultured dorsal root ganglion (DRG) neurons (4). Etanercept (Enbrel®, Amgen, Inc., Thousand Oaks, CA), a diametric fusion protein consisting of the TNF receptor II (TNFRII) and the constant portion of human IgG, can competitively bind TNF to attenuate neuropathic pain (5). However, little is known about the effect of Etanercept on nerve regeneration. In the present study, we examined the patterns of TNF mRNA expression levels throughout the course of WD after rat sciatic nerve crush and evaluated the effect of Etanercept on axonal regeneration.

Materials and Methods: Surgical Procedure. A total of 98 adult female Sprague-Dawley rats (Harlan Labs, Indianapolis, IN) were used. The left sciatic nerve was exposed and crushed using smooth-surface forceps once for 5 seconds.

Real-time qPCR. Two sciatic nerve and L5 DRG samples were pooled for TNF mRNA analyses at 1, 5, 7, 14, 28, and 60 days after crush injury. Total RNA was extracted with Trizol (Invitrogen, Carlsbad, CA). TNF gene expression was measured by qPCR (MX4000, Stratagene, La Jolla, CA). Primers and Taqman probes were synthesized by Biosearch Technologies (Novato, CA). Relative mRNA levels were normalized to Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) using the comparative Ct method. Data were expressed as a fold change in TNF mRNA in injured compared with naive nerve and analyzed for statistical significance using ANOVA followed by Tukey’s post-hoc test (p value <0.05).

Systemic or local Etanercept therapy. Etanercept or sterile bacteriostatic water (vehicle) was administered (1) intraperitoneally or (2) into the epineurial space adjacent to the crushed nerve. Animals were given three doses of Etanercept (0.3, 3.0, 6.0 mg/kg) once (immediately after the crush injury) or twice (1 hour and 3 days after the crush injury).

Pinch testing. Axonal regeneration in rats was evaluated using the pinch test at day 5 following nerve crush. Pinch tests were performed in the crushed animals with systemically or locally administered vehicle (n=5, each group) or Etanercept (0.3, 3.0, 6.0 mg/kg, n=5 to 6, each group). The regeneration distance between this pinch site and the stitch marking the original crush site was measured. Data were analyzed for statistical significance using the Mann-Whitney U-test (p value <0.05).

Histology for neuropathologic evaluation. Histologic examinations were performed in the crushed animals with systemically treated vehicle or Etanercept (6.0 mg/kg) (n=2, each group, at 1, 3, 5 days after crush). The segments of the nerve which sectioned axially 10 mm distal to the original crush site were removed and embedded in araldite. One-micron-thick sections were stained with methylene blue and Azure II.

Results: TNF mRNA expression in crushed nerve and corresponding DRG. TNF mRNA expression in the crushed nerve (p<0.001) and corresponding DRG (p<0.05) was significantly elevated at 1 day after crush injury and subsequently returned to baseline by 5 days after crush injury (Figure 1A).

Etanercept enhances axonal regeneration of rat sciatic nerve. In the highest (6.0 mg/kg) dose of Etanercept-treated animals, the regeneration rate was significantly improved in both systemic (p<0.01) and local (p<0.05) administration as compared with vehicle-treated animals at 5 days after nerve crush injury (Figure 1B).

Neuropathologic changes of regenerating nerve. Histological examination of nerve tissue from the distal fragments of the crush site, in both vehicle- and Etanercept-treated groups, demonstrated extensive WD. At 5 days after crush injury, there are striking differences in endoneurial regenerative nerve clusters, shown as small white structures in the figure (Arrow; Figure 2). Although not quantified, in the Etanercept-treated group, there appeared to be an increase in small bundles of regenerated nerve fibers as compared with vehicle-treated animals.

Discussion: In the present study, we demonstrated that TNF mRNA expression in nerve and corresponding DRG were up-regulated as early as 1 day after rat sciatic nerve crush and subsequently returned to baseline by 5 days, and acute treatment with Etanercept enhanced axonal regeneration. Functional nerve regeneration requires axonal regrowth coupled with a local permissive environment. TNF inhibits axonal outgrowth in cultured DRG neurons (4). Following nerve injury, macrophages that are recruited to the injury sites contribute to debris clearance, which generates a permissive environment for regeneration. In TNF-knock-out mice, the number of macrophages during WD was significantly reduced, while myelin phagocytosis was not affected (6). Thus, acute treatment with Etanercept may affect axon outgrowth without delayed myelin debris clearance after nerve injury. Our results offer a promising new treatment approach for peripheral nerve injury.


Figure 1A: TNF mRNA expression in crushed sciatic nerve and ipsilateral L5 DRG, determined by real-time qPCR, using GAPDH as a normalizer. Data are expressed as the fold increase relative to naïve animals. Each value represents mean +/- SE of 5 to 7 samples. B: Systemically and locally administered Etanercept into crushed nerve enhanced functional regeneration of rat sciatic nerve. Regeneration distances were measured using pinch test 5 days after crush. Data are expressed as means +/- SE.

Figure 2: Neuropathologic changes of regenerating nerve after systemic administration of Etanercept at 5 days after crush injury. Arrow indicates small bundles of regenerating nerve fibers.