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Assessment of serum-mediated neurotoxicity in Navajo neuropathy

M. W. Lawlor¹, S. Holve² and E. B. Stubbs Jr.³

Abstract

Navajo neuropathy is a unique sensorimotor neuropathy which is geographically restricted to Navajo children living on the Navajo Reservation. Affected patients present with weakness, loss of sensation in extremities, corneal ulcerations, and a high incidence of childhood infections. Metabolic complications, such as severe liver disease, may further contribute to peripheral nerve injury in affected patients. In this study, serum-mediated injury to rat peripheral nerve was critically assessed. Serum samples from affected Navajo patients were tested in vivo for effects on peripheral nerve function. Injection of serum from affected Navajo patients into rat sciatic nerve produced a modest slowing of nerve conduction velocity without effecting evoked-compound muscle action potential (CMAP) amplitudes. By comparison, injection of serum from patients with MGUS neuropathy, an immune-mediated disorder, diminished evoked-CMAP amplitudes by approximately 70%. Navajo neuropathy sera had no effect in vitro on the neurite outgrowth of developing dorsal root ganglia neurons. The results argue against serum-mediated toxic injury to peripheral nerves in Navajo neuropathy.

Key-words: Nerve – Neuropathy – Intraneural injection – Navajo – Liver.

Introduction

A geographically restricted sensorimotor neuropathy affecting children living on the Navajo Reservation in southwestern United States has been previously reported (1). This autosomal recessive disorder, termed Navajo neuropathy, is manifested by neurological deficits which resemble hereditary sensory neuropathy type II with additional findings that include severe motor disorder, corneal ulceration, and high levels of CSF protein. Mental function and intelligence are normal. Navajo neuropathy occurs with an incidence similar to that of cystic fibrosis, affecting 1 in

2,200 live births in its target population (12). Navajo neuropathy is predominantly seen on the western portion of the Navajo Reservation, a remote area with restricted access.

Environmental factors including heavy metals, water source, toxin exposure, and family occupation have been excluded as etiologic agents of this disorder (12). Liver disease can be a contributing factor in the pathogenesis of some peripheral neuropathies and is frequently observed in patients with Navajo neuropathy (1, 12). Hepatic neuropathies may result from metabolic derangement (15) or toxins secondary to hepatocellular damage (2-5). The possibility that serum-born toxin(s) elicit peripheral nerve injury in Navajo neuropathy has not been previously studied.

In this study we used intraneural injection (11, 17) as a direct method to measure serum effects in vivo on rat peripheral nerve function. Sural nerve biopsy specimens from these patients were not available for study. A selective loss of myelinated fibers in

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sural nerves of affected Navajo children has, however, been documented (1). In the same study, Appenzeller et al. reported that degeneration of myelinated fibers was clearly evident in younger patients. Onion bulb formation, a morphological indicator of repetitive demyelination and remyelination, was absent. These findings suggest an inability of affected peripheral nerves to remyelinate. There was no evidence to support regeneration of myelinated fibers. Unmyelinated axons were reported morphologically normal.

Materials and methods

Serum samples from five Navajo children with peripheral neuropathy and liver disease (affected) or from ten asymptomatic (unaffected) siblings were generously provided by Dr. George DeVries (Loyola University Chicago, Maywood, IL). A fixed volume (5 μ l) of serum from each patient, supplemented with an equal volume of fresh human or rabbit complement (7, 10, 13), was injected into the endoneurium of rat sciatic nerve 10 mm distal to the sciatic notch as previously described (11). Nerve conduction latency and evoked compound muscle action potential (CMAP) amplitudes were measured prior to and 5 days following intraneural injection (19). Proximal (sciatic notch)- and distal (ankle)-evoked responses were evoked using supramaximal stimuli (25 mA) and recorded from the plantar muscle of the appropriate hind limb using unipolar pin electrodes with a reference electrode placed on the third digit. Body temperature was maintained at 37°C with a heating pad. Evoked potentials were captured and analyzed with an Advantage electromyograph. Sciatic nerve conduction velocity was calculated by dividing the distance (typically 55 mm) between the points of stimulation by the difference in the onset latencies (typically 1 ms) of the evoked negative peak amplitudes. Nerve conduction across the injection site was measured as a ratio of proximal- to distal-evoked CMAP amplitudes (14).

Results and discussion

To evaluate quantitation of nerve injury *in vivo*, the right sciatic nerve of untreated sodium pento-

barbital anesthetized rats were subjected to a 30 s crush 10 mm distal to the sciatic notch. Evoked motor responses recorded prior to and at 5 days after experimental crush are shown in Figure 1. Experimental crush of sciatic nerve markedly reduced proximal-evoked CMAP amplitude by greater than 90%. Evoked amplitudes recorded distal to the crush injury were similar to pre-crush values (5.9 ± 0.7 mV, $n = 5$), consistent with a proximal conduction block. After 5 days, both proximal- and distal-evoked motor responses were reduced to less than 10% of control, suggesting Wallerian degeneration of the sciatic nerve.

The effect of serum from Navajo patients on sciatic nerve function *in vivo* was next assessed. Intraneural injection of serum samples from affected Navajo children slowed sciatic nerve conduction velocity to 42.3 ± 6 m/s ($n = 5$) compared with injection of sera from asymptomatic siblings (56.1, $n = 2$) or from non-injected rat sciatic nerve (54.8 ± 4 , $n = 5$). This sera, however, had no detectable effect on proximal- or distal-evoked CMAP amplitudes of rat nerve (Fig. 1). By comparison, intraneural injection of serum from a patient with MGUS neuropathy, an immune-

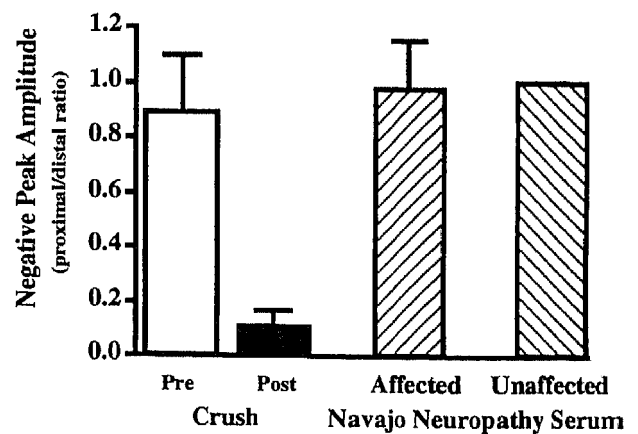


Fig. 1. - Evoked-motor responses obtained *in vivo* from rat sciatic nerve injected with sera from affected Navajo children. Sera from 5 Navajo patients with neuropathy (affected) or from 2 asymptomatic relatives (unaffected) were independently assessed. Compound muscle action potential amplitudes were recorded before and 5 days after intraneural injection and are expressed as a ratio of proximal-to-distal CMAP amplitudes. Data shown are the mean \pm SD (affected) or the average (unaffected) of motor responses obtained. Results of sciatic nerve crush (mean \pm SD, $n = 3$), representative of two independent experiments, are shown for comparison.

mediated disorder (6, 8, 9), reduced proximal-evoked CMAP amplitudes $70 \pm 5\%$ ($n = 3$) compared with serum from an age- and sex-matched healthy volunteer. The paucity of myelinated fibers in Navajo neuropathy may result from progressive demyelination with secondary axonal loss or from primary axonal degeneration. Sural nerve biopsy from younger affected Navajo patients supported a degenerative, rather than demyelinating, pathological process (1).

To directly test for an effect of sera on nerve growth, neurons from E15 rat dorsal root ganglia (DRG) were cultured (18) in growth medium supplemented (10%) with sera from five affected Navajo patients or from ten asymptomatic relatives for 6 days and neurite outgrowth was assessed by light microscopy. Sera from affected Navajo patients altered neither DRG neurite outgrowth or the morphology of developing neurites (Fig. 2). By comparison, serum from a patient with MGUS neuropathy inhibited neurite outgrowth by greater than 50% (Dr. Thomas Lopez, personal communication).

The findings from this study show that sera from affected Navajo children, when injected into rat sciatic nerve, produce a modest slowing of nerve conduction velocity without affecting proximal- or distal-evoked CMAP amplitudes. These results are

consistent with the morphometric observations of Appenzeller et al. (1), suggesting that peripheral nerve injury in Navajo neuropathy occurs as a result of a chronic process more consistent with primary axonal degeneration rather than acute demyelination. The absence of an acute effect of sera on the growth of developing neurons in culture argues against a serum-mediated metabolic neurotoxicity reported in some patients with liver disorders (2-5). Species selectivity or the maturational stage of DRG neurons used in this study remain, however, possible confounding influences. Recurrent systemic infections seen in affected Navajo children raise the possibility of immunologic abnormalities (12). Hereditary sensory neuropathy, similar to Navajo neuropathy, has been associated with an increase in serum immunoglobulin content (16). No statistical differences were seen, however, in the content of immunoglobulins G or M in serum samples from five affected Navajo children compared to ten asymptomatic relatives (mg/dl of IgG: $1,267 \pm 231$ vs. $1,495 \pm 372$; mg/dl of IgM: 136 ± 40 vs. 128 ± 56 , affected vs. unaffected respectively). Nerve injury associated with monoclonal gammopathy is therefore an unlikely etiology of Navajo neuropathy. The results of this study argue against the presence of neurotoxic constituents in sera of affected Navajo children.

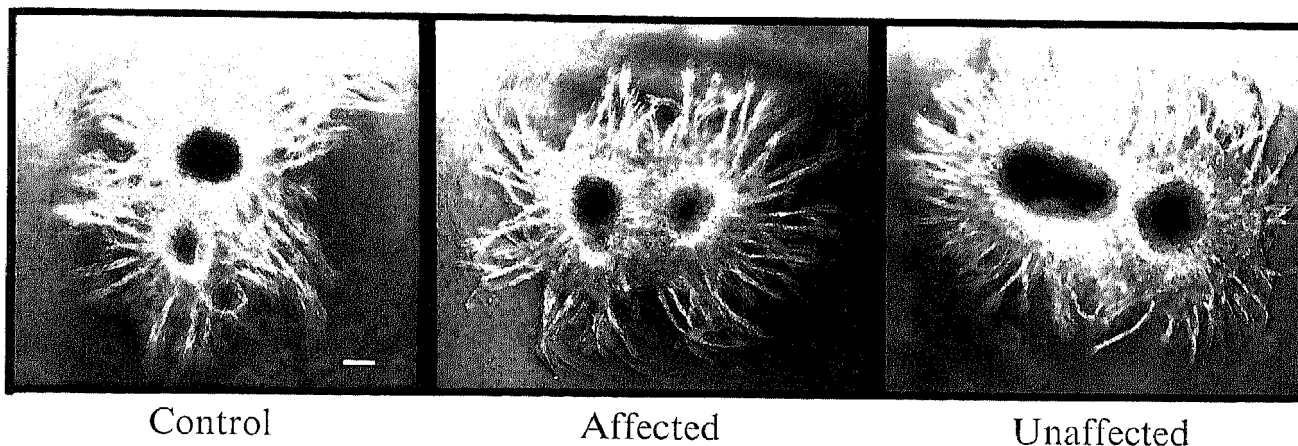


Fig. 2. - Representative photographs of developing rat E15 dorsal root ganglia (DRG) neurons cultured for 6 days in media supplemented with 10% fetal bovine sera (control, $n = 5$) or 10% sera from affected ($n = 10$) or unaffected ($n = 5$) Navajo children. Bar, 1.5 mm.

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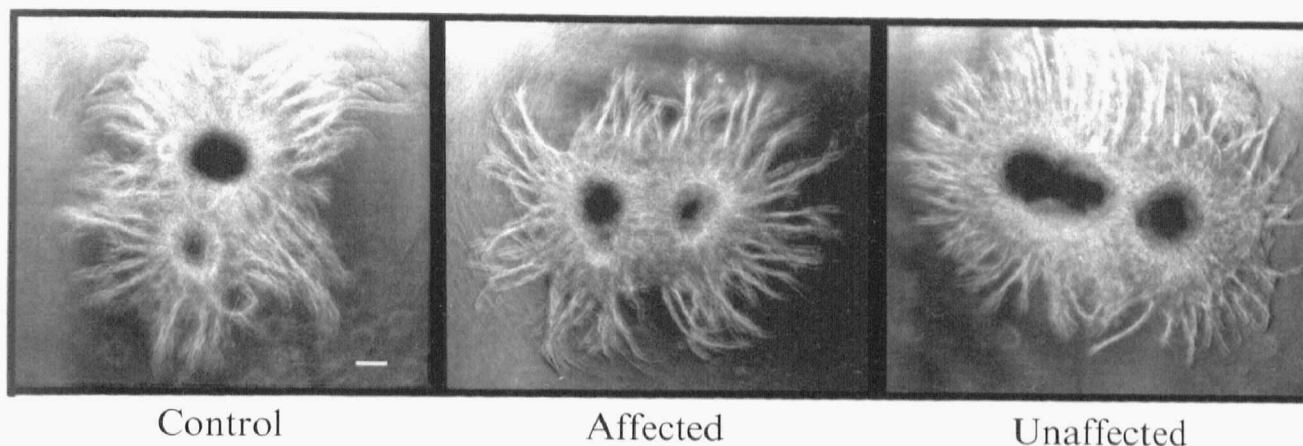


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