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Paclitaxel alters sensory nerve biomechanical properties

Brian G. Bober [a], Sameer B. Shah [a,b,*]

[a] Department of Bioengineering, University of California, San Diego, La Jolla, CA, USA
[b] Department of Orthopaedic Surgery, University of California, San Diego, La Jolla, CA, USA

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ABSTRACT

Paclitaxel is an effective chemotherapeutic that, despite its common use, frequently causes debilitating peripheral sensory neuropathy. Paclitaxel binds to and stabilizes microtubules, and through unknown mechanisms, causes abnormal microtubule aggregation. Given that microtubules contribute to the mechanical properties of cells, we tested the hypothesis that paclitaxel treatment would alter the stiffness of sensory nerves. Rat sural nerves were excised and soaked in Ringer's solution with or without paclitaxel. Nerves were secured between a force transducer and actuator, and linearly strained. Stress–strain curves were generated, from which elastic moduli were calculated. Paclitaxel treated nerves exhibited significantly higher moduli in both linear and transition regions of the curve. A composite-tissue model was then generated to estimate the stiffness increase in the cellular fraction of the nerve following paclitaxel treatment. This model was supported experimentally by data on mechanical properties of sural nerves stripped of their epineurium, and area fractions of the cellular and connective tissue components of the rat sural nerve, calculated from immunohistochemical images. Model results revealed that the cellular components of the nerve must stiffen 12x to 115x, depending on the initial axonal modulus assumed, in order to achieve the observed tissue level mechanical changes. Consistent with such an increase, electron microscopy showed increased microtubule aggregation and cytoskeletal packing, suggestive of a more cross-linked cytoskeleton. Overall, our data suggests that paclitaxel treatment induces increased microtubule bundling in axons, which leads to alterations in tissue-level mechanical properties.

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1. Introduction

Paclitaxel is an effective and common chemotherapeutic that prevents cell division by stabilizing microtubules, thus preventing their depolymerization(Amos and Lowe, 1999). Due to its systemic delivery, paclitaxel also accumulates in peripheral nerves, causing a debilitating sensory neuropathy(Argyriou et al.,2014). Because neurons are post-mitotic, mechanisms underlying paclitaxel-induced neuropathy must differ from those affecting dividing cells. Animal models of disease and patients display dying back of axons, reduced epidermal nerve fiber density(Boyette-Davis et al.,2011), demyelination(Argyriou et al.,2008), altered ion channel activity(Hara et al.,2013), and disruptions in axonal transport. The latter pathway is commonly hypothesized to underlie neuropathic progression; abnormal clustering of microtubules(Masurovsky et al.,1981; Masurovsky et al.,1983; Turner and Margolis,1984) is thought to impair delivery of vital proteins and organelles by microtubule-based motor complexes to distal axonal reaches.

Beyond their role in transport, microtubules also play important structural and biomechanical functions in axons (Garcia et al., 2012; Ouyang et al., 2013; Peter and Mofrad, 2012), which may also be perturbed by paclitaxel. For example, a recent study demonstrated, using atomic force microscopy, that paclitaxel increases the compressive modulus of cell bodies in cultured dorsal root ganglia (Au et al., 2014). Paclitaxel also alters the response of central neurons to damage during high strain-rate tensile loading (Tang-Schomer et al., 2010), and theoretical studies suggest that microtubules may influence tensile axonal biomechanics (Garcia, Pena et al., 2012; Peter and Mofrad, 2012).

Whether and how structural and mechanical changes caused by paclitaxel influence biomechanics and physiological function at the tissue level has not been tested. Tensile loading is particularly relevant in peripheral nerves, which span articulating joints and can incur substantial regional strains (Aoki et al., 2005; Ochi et al., 2013; Topp and Boyd, 2006). However, deformation beyond normal levels impairs action potential conduction (Wall et al., 1992). To protect neurons, strain is partially accommodated by axonal undulations, which straighten upon

* Corresponding author at: 9500 Gilman Drive, MC 0863 La Jolla, CA 92093, USA. Tel.: +1 858 822 0720. fax: + 1 858 822 3807.
E-mail address: sbshah@ucsd.edu (S.B. Shah).

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nerve deformation (Haninec, 1986; Rydevik et al., 1990). In axons themselves, maintenance of structural integrity during axonal straightening and subsequent lengthening is likely to be modulated by the cytoskeleton, including microtubules. Increasing microtubule amount, length, stiffness, and bundling through paclitaxel treatment could alter the mechanical response and stability of this delicate biomechanical system, leading to impaired function. This study uses a sensory nerve model to test the hypothesis that paclitaxel causes changes in the tensile properties of peripheral nerves. Our data suggest that paclitaxel modifies the structure and biomechanics of sensory nerves, supporting a new hypothesis that altered neuronal biomechanics could contribute to paclitaxel-induced sensory dysfunction.

2. Methods

2.1. Mechanical testing

12–20 week old Sprague–Dawley rats were sacrificed and sural nerves were exposed and freed of the surrounding connective tissue bilaterally. A 5 mm sural nerve segment just distal to the trifurcation point of the sciatic nerve was tied off with 6-0 nylon sutures *in situ* (Fig. 1a). Both nerve segments were excised and randomly assigned to be soaked in Ringer's solution (Aqueous solution of NaCl: 120 mM, KCl: 4 mM, $MgSO_4$: 0.8 mM, $NaHCO_3$: 16 mM, glucose: 20 mM, and $CaCl_2$: 2.2 mM, brought to pH 7.4) with or without the addition of 20 μM paclitaxel, for 2 h. Each nerve segment was attached to an Aurora 402A force transducer and linear actuator and stretched in a chamber containing the appropriate Ringer's solution.

The length at which the nerve first incurred detectable passive load was recorded as L_0. Nerves were stretched in increments of 0.2–0.5 mm at a rate of ~0.05 mm/s and held for 5 min before recording the force output, to allow stress relaxation (Luna et al., 2013; Shah and Lieber, 2003). Because sutures securing the nerve to the force transducer and actuator were initially slack, nerve length was measured from suture to suture from images captured at each incremental deformation (Fig. 1b). All measurements were made blinded as to paclitaxel treatment. We alternately normalized lengths to the length at which nerve stress was equal to 2 g-force/mm^2 ($L_{\sigma=2}$). Cross-sectional area was calculated from nerve diameters measured from images captured at L_0, assuming the nerve as a cylinder. Engineering stress–strain (σ–ε) curves were then generated, normalizing to L_0 or $L_{\sigma=2}$. Segmental strains were also measured using ink dots along the nerve, to determine the eventual region of failure.

To test the mechanical properties of nerves without their epineurium, sural nerves were excised as above. The tip of the inner sheath, which extruded beyond the epineurial boundary (Walbeehm et al., 2004), was held with forceps and the epineurium was manually peeled away. Mechanical testing was performed as above.

The elastic modulus of each curve was calculated as the slope of the linear region of each stress–strain curve at high strains. To compare tangent moduli at specific lower strains preceding the linear region, each stress–strain curve, with strain normalized to $L_{\sigma=2}$, was plotted from $\varepsilon=0$ to the start of the linear region, and fit with an exponential function $\sigma=A^*\exp(B^*\varepsilon)$ as for other biological tissues (Xiong et al., 2008; Yamanari et al., 2012). $A=2$, such that $\sigma=2$ g-force/mm^2, by definition, at $\varepsilon=0$. All curves had R^2 values $>.95$. Tangent moduli of non-linear regions of each curve were then calculated by the derivative of the exponential curve fit with respect to strain. The strain at which the tangent modulus of the exponential equation equaled the slope in the linear region was denoted as ε_L. The region of the stress–strain curve with $\varepsilon < \varepsilon_L$ are referred to as the transition region.

Because our higher-resolution mechanical testing apparatus was not designed to measure forces larger than 50 g-force, we performed additional testing on a separate apparatus to measure the ultimate stress of 5 pairs of sural nerves. Contralateral pairs of ~5 mm segments of sural nerves were bathed in Ringer's solution with or without paclitaxel as above, mounted between a force transducer (Omega, DFG55-5) and a linear actuator, and strained in increments of 0.5 mm every 30 s. Maximum force at failure was recorded, and ultimate stress was calculated by dividing the force by the resting cross sectional area of the nerve.

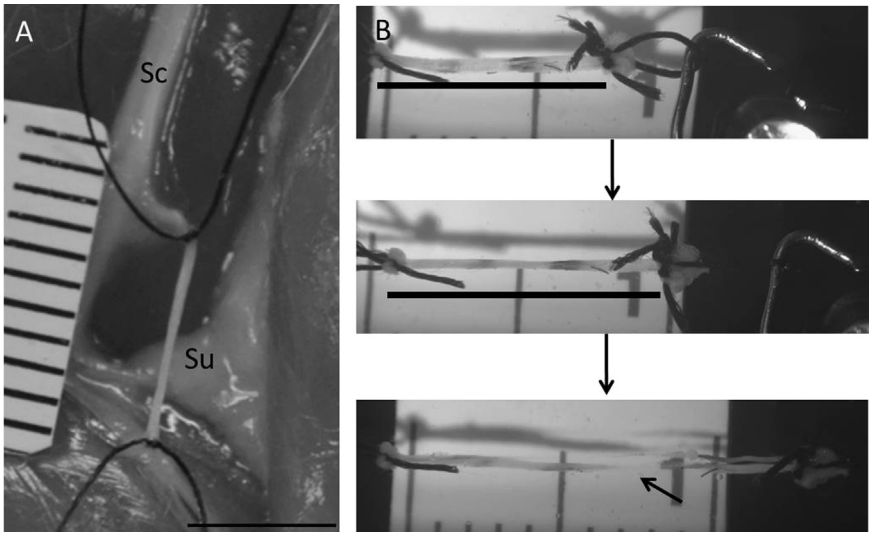

Fig. 1. Mechanical testing of sural nerves. A) Sural nerves were exposed in rat cadavers. A ~5 mm segment of each nerve immediately distal to the trifurcation point of the sciatic nerve was sutured *in situ* with 6-0 nylon sutures. Sc and Su indicate the sciatic and sural nerves, respectively. Scale bar=5 mm. B) Each nerve was tied to a force transducer and a linear actuator, and progressively strained over time until visible nerve rupture (arrow). Ink dots along the nerve were used to monitor regional deformation.

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