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Regular Article

Independent evaluation of the anatomical and behavioral effects of Taxol in rat models of spinal cord injury

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ABSTRACT

The goal of the current manuscript was to replicate published data that show intrathecal infusions of Taxol® (paclitaxel), an anti-neoplastic microtubule stabilizing agent, reduce fibrogliotic scarring caused by a dorsal spinal hemisection (DHx) injury and increase functional recovery and growth of serotonergic axons after moderate spinal contusion injury. These experiments were completed as part of an NIH-NINDS contract entitled "Facilities of Research Excellence in Spinal Cord Injury (FORE-SCI) — Replication". Here, data are presented that confirm the anti-scarring effects of Taxol after DHx injury; however, Taxol did not confer neuroprotection or promote serotonergic axon growth nor did it improve functional recovery in a model of moderate spinal contusion injury. Thus, only partial replication was achieved. Possible explanations for disparate results in our studies and published data are discussed.

Introduction

Following traumatic spinal cord injury (SCI), microtubule stability is necessary for a wide range of responses, including cell survival, cell proliferation, migration of peripheral cells and glia, intracellular signaling and axon transport, and growth of injured and spared axons. Paclitaxel (Taxol®) is an FDA-approved anti-cancer drug that stabilizes microtubules and inhibits mitotic spindle assembly (Vyas and Kadow, 1995). Recent data indicate that this drug could also be a neuroregenerative therapy. Indeed, Taxol can prevent axon retraction, enhance axon growth, reduce leukocyte infiltration and migration and reduce fibrotic scarring, in part by inhibiting cell proliferation and secretion of extracellular matrix (ECM) molecules (Ertürk et al., 2007; Hellal et al., 2011; Sengottuvel et al., 2011).

In a recent publication, Hellal et al. showed that infusion of low dose Taxol at the site of SCI reduced glial and mesenchymal scar formation and increased numbers of serotonergic (5HT) axons below the lesion. These anatomical changes were associated with improved behavioral recovery in a rat spinal cord contusion injury model (Hellal et al., 2011). In consideration of the promising translational potential for this drug, the present study was performed to provide independent replication of the original findings, under the guidelines of a Facilities of Research Excellence in Spinal Cord Injury (FORE-SCI) contract with the National Institute of Neurological Disorders and Stroke (NINDS). For this experiment, we attempted to replicate the primary histological and/or behavioral effects of Taxol infusion for 7 days following a mid-thoracic dorsal hemisection (DHx) or for 28 days following a moderate mid-thoracic spinal contusion injury. Here, partial replication was achieved. Specifically, the anti-scarring effects of Taxol were confirmed in both models of SCI; however, Taxol did not affect serotonergic axon density below the lesion, nor did it improve functional recovery in a model of spinal contusion injury.

Abbreviations: SCI, spinal cord injury; dpi, days post-injury; ECM, extracellular matrix; CSPG, chondroitin sulfate proteoglycan; DHx, dorsal hemisection; 5HT, 5-hydroxytryptamine (serotonin); NIH, National Institutes of Health; NINDS, National Institute of Neurological Disorders and Stroke; FORE-SCI, Facilities of Research Excellence in Spinal Cord Injury; EC/CV, Eriochrome® cyanine/cresyl violet; MCID, microcomputer imaging device; BBB, Basso–Beattie–Bresnahan locomotor rating scale; GFAP, glial fibrillary acidic protein.

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Materials and methods

All methods and data were reported with consideration of guidelines provided by *Animals in Research: Reporting in Vivo Experiments* (*ARRIVE*) and *Minimum Information About a Spinal Cord Injury Experiment* (*MIASCI*) (Kilkenny et al., 2010; Lemmon et al., 2014).

Animal group sizes, power calculations and group designations

The effort to replicate Hellal et al. was completed in two phases. Phase 1 targeted Fig. 1 from Hellal et al. in which Taxol was shown to reduce scarring in a rat hemisection lesion model (Hellal et al., 2011). Phase 2 was designed to replicate Hellal's Figs. 4E & F, where Taxol was shown to increase 5HT axon labeling below the site of injury and improve behavioral recovery after spinal contusion injury. As the goal was to examine the potential for translation, we were not charged to replicate data in Figs. 4A–D, which show that Taxol improves axon growth following a peripheral conditioning lesion.

Prior to starting phase 1, n = 13 animals were used in pilot studies to establish consistent injury technique and catheter placement. No quantitative data were generated from these animals. For replication of Fig. 1, animal group sizes were matched to those described in the original manuscript (n = 14/group). Before attempting to replicate Figs. 4E & F, optimal group sizes were calculated via power analyses. Using raw data from Fig. 4E only (anatomical analysis of 5-HT+ fibers) it was determined that a 50% increase in the number of 5HT+ fibers would yield power of 0.8 with $\alpha = 0.05$ using n = 7 rats/group. However, using raw data from Fig. 4F (provided by the original authors), power calculations for a 2 way ANOVA indicated that hundreds of animals/group were necessary to sufficiently power a replication of the effects of Taxol on behavioral recovery. This was impractical under the auspices of the replication contract, so logistics (e.g., timing, expense) and past experience

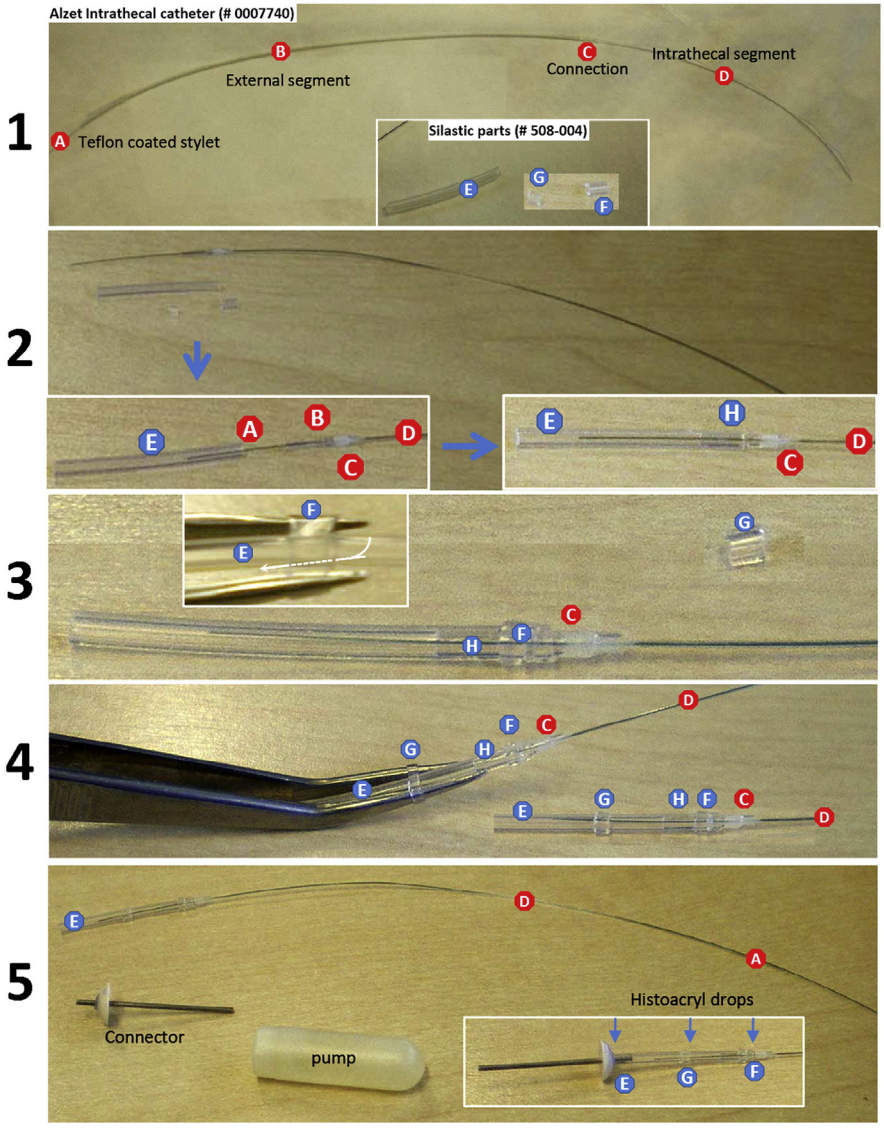

Fig. 1. Custom designed catheters for intrathecal delivery of cremophor vehicle and/or Taxol. A rat intrathecal catheter (Alzet #0007740) was modified by inserting a segment of Silastic tubing at the junction between the osmotic pump and the remaining segment of intrathecal catheter. (1) Briefly, intrathecal catheters, comprised of a thin Teflon-coated wire stylet (A), an external catheter segment (B), a connector segment (C) and the intrathecal segment (D) were cut to a length of ~50 mm. Three separate pieces of Silastic tubing (Dow Corning; #508-004) also were prepared for each catheter; two cuffs (4 mm and 2 mm) and a single 20 mm segment (E, F, G). (2) The external segment of the catheter is cut to a length of ~8 mm (B) then is inserted along with the stylet (A) into one end of the 20 mm segment of Silastic tubing (E) until it abuts the connector (C) just proximal to the distal catheter segment (D). (3) A 4 mm Silastic cuff (F) was slipped over this junction (H) then the external catheter segment was connected to the metal hub of the osmotic pump. (4) The remaining 2 mm Silastic cuff (G) was placed midway over the distal 20 mm segment of Silastic tubing where it serves as an anchor point for securing sutures on each side of the cuff. (5) Finally, a few drops of Histoacryl® (TissueSeal, LLC; #TS1050071FP; Ann Arbor, MI) was applied to all cuffs (F, G) and at the interface between the long segment of Silastic tubing (E) and the pump connector. This seals all connections and prevents leakage.