Microtubule Stabilization Reduces Scarring and Causes Axon Regeneration After Spinal Cord Injury

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Hypertrophic scarring and poor intrinsic axon growth capacity constitute major obstacles for spinal cord repair. These processes are tightly regulated by microtubule dynamics. Here, moderate microtubule stabilization decreased scar formation after spinal cord injury in rodents through processes during scarring, including cell proliferation, migration, and differentiation as well as intracellular trafficking and secretion of extracellular matrix (ECM) molecules (1, 2). Moreover, moderate microtubule stabilization prevents axonal retraction and swelling of the axon tip after central nervous system (CNS) injury (3) and stimulates axon growth of cultured neurons (4), enabling them to overcome the growth inhibitory effect of CNS myelin (3). We hypothesized that moderate microtubule stabilization with Taxol, an approved drug, would facilitate axonal regeneration after spinal cord injury (SCI) by decreasing scar formation and enhancing intrinsic axonal growth.

We first examined whether Taxol treatment reduced scarring after SCI. Adult rats underwent a dorsal hemisection at the eighth thoracic spinal cord level; Taxol was continuously delivered at the injury site using an intrathecal catheter connected to an osmotic minipump. Seven days after injury, the lesion of vehicle-treated animals was filled with laminin, fibronectin, and collagen IV, hallmarks of a fibrotic scar (Fig. 1, A and B, and fig. S1), which is a major impediment for axon regeneration (5–7). In contrast, in the lesion of animals treated with Taxol at 256 ng/day, a much lower dose than used for chemotherapy (8), laminin, fibronectin, and collagen IV were strongly reduced (Fig. 1, C and D, and fig. S1). At this Taxol concentration, astrocytes surrounded the injury as in vehicle controls (Fig. 1, B, C, and E); the injury size was equivalent in both groups (Fig. 1F), which suggests that astrocytes isolated the lesion from undamaged CNS tissue (9). Phosphohistone-H3 immunostaining and terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick end labeling (TUNEL) showed that the numbers of proliferating and apoptotic cells were similar between Taxol- and vehicle-treated animals (fig. S2), which means that at low doses, Taxol reduced fibrotic scarring by

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mechanisms independent of cell proliferation or apoptosis.

A key event in fibrotic scarring after CNS injury is the activation of transforming growth factor-β (TGF-β) signaling. Following SCI, TGF-β expression dramatically increases, which favors fibrosis (10–12). Integrity of the microtubule network is crucial for the transduction of this signal (13). Smad2, the downstream effector of the TGF-β pathway, binds to microtubules through conventional kinesin-1 (14). We asked whether stabilizing the microtubule network hinders TGF-β signaling and attenuates fibrogenesis. In extracts from the injury site, Taxol treatment increased the level of detyrosinated microtubules (Fig. 2A), enabling kinesin-1 to bind tightly to microtubules (15). Indeed, in Taxol-treated animals, kinesin-1 was enriched in the microtubule fraction of the injury site extracts (Fig. 2B). In brain and spinal cord extracts, Smad2 directly bound to kinesin-1 (Fig. 2, C and D). Taxol strongly altered both kinesin-1- and dynein-driven cargo transport in an intracellular trafficking assay (16) (Fig. 2, E and F), suggesting that Taxol would hinder Smad2 trafficking. Indeed, in TGF-β-stimulated astrocytes, Taxol caused Smad2/3 to localize persistently to microtubules (fig. S4) and inhibited 70% of its translocation to the nucleus (Fig. 2, G and H). In time-lapse microscopy, overexpressed Smad2 fused to photoactivatable green fluorescent protein (PAGFP) moved into the nucleus within minutes after TGF-β1 stimulation, whereas Taxol treatment abolished this movement (movies S1 and S2). In vivo, 7 days after SCI, phosphorylated Smad2/3 translocated into the nucleus in 95% of vehicle-treated animals, compared with only 30% of the Taxol-treated animals (n = 13 animals per group) (Fig. 2I). This suggested that Taxol impairs TGF-β signaling-dependent processes. Indeed, in cultured meningeal cells, Taxol reduced the TGF-β1–stimulated production of fibronectin (Fig. 3A) and impaired TGF-β1–stimulated migration (Fig. 3, B and C). Thus, low doses of Taxol prevent fibrotic scarring after SCI by interfering with Smad-dependent TGF-β signaling and reducing extracellular matrix secretion and cell migration.

TGF-β signaling also regulates the production of the axon growth inhibitory chondroitin sulfate

![Fig. 2. Taxol dampens TGF-β signaling.](image-url)

(A) Taxol treatment increases total tubulin and decreases tyrosinated tubulin in the lesion site. (B) Kinesin-1 enrichment in microtubule fraction of Taxol-treated lesion site. (C) His-Smad2 binds to kinesin-1 and endogenous Smad2 communoprecipitates with kinesin-1 (D) of brain and spinal cord extracts. (E and F) Taxol alters microtubule-based cargo transport. (E) Overlay of color-coded time series of red fluorescent protein (RFP)–labeled peroxisomes bound to the kinesin-1 (KIF5) motor domain upon Rapalog addition. Blue marks the initial distribution; the red gradient shows the distribution over time (30 min). Scale bars, 10 μm. (F) Time traces of radius of circle enclosing 90% of total fluorescence intensity for KIF5- or dynein adaptor (BICDN)–linked peroxisomes. Mean ± SEM of 5 to 8 COS-7 cells per condition (P = 0.004; two-tailed t test for both KIF5 and BICDN). (G and H) In cultured astrocytes, Taxol counteracts the TGF-β1–induced nuclear translocation of Smad2/3 (arrowheads) causing cytoplasmic Smad2/3 accumulation (arrow). Results in (H) are mean ± SD [three independent experiments; *P = 0.041; one-way analysis of variance (ANOVA)]. (I) At 7 days after injury, Taxol treatment interferes with the nuclear translocation of phospho-Smad2/3 induced by SCI (n = 13 rats per group). Scale bar, 20 μm. BSA, bovine serum albumin; DAPI, 4′,6′-diamidino-2-phenylindole; DMSO, dimethyl sulfoxide.
proteoglycans (CSPGs) (10). We asked whether Taxol decreases CSPGs after SCI. At 7 days after injury, Taxol decreased the amount of NG2, one of the most abundant CSPGs (17) (fig. S1). The inhibitory effects of CSPGs on axon growth reside in their Glycosaminoglycan (GAG) side chains (18–20). Lesion site extracts from Taxol-treated animals showed a significant reduction of GAGs compared with controls (Fig. 3D). The conditioned medium of cultured meningeal cells and astrocytes treated with 10 nM Taxol showed a 35% and 32% decrease of GAG levels, respectively (Fig. 3, E and F). Moreover, the CSPGs expressed in the Taxol-treated animals localized to the intracellular space instead of scaffolding the cells as observed in vehicle-treated animals (Fig. 3G). Thus, administration of low doses of Taxol decreases CSPGs at the lesion site after SCI.

We next asked whether the Taxol-treated lesion site becomes permissive for regenerating axons in vivo by evaluating the regenerative response of dorsal root ganglion (DRG) neurons. These neurons are set into a growth-competent state by injuring their peripheral axon (conditioning) that allows them to regenerate their CNS axon, but only in a scar-free environment (21). We assessed whether the reduction of the scar induced by Taxol is permissive for conditioned axons to grow. Taxol was delivered at the lesion for 4 weeks; 2 weeks after central injury, we conditioned the lumbar L4-6 DRG neurons by transecting the sciatic nerve. Of the vehicle-treated animals, 76% had no regenerative response but rather showed fiber retraction (21) (Fig. 4A). In contrast, Taxol-treated animals had regenerative fibers growing along the edge of the lesion cavity into the injury site and beyond (Fig. 4A). The longest axons per animal grew 1199 ± 250 μm in the Taxol-treated group versus 176 ± 225 μm in the vehicle-treated animals (n = 13 animals per group; P = 0.002; two-tailed t test) (Fig. 4B). The Taxol-treated lesion site thus becomes favorable for regeneration of growth-competent axons.

Because Taxol also enhances intrinsic axon growth (4) and the elongation of cultured neurons plated on CSPGs or CNS myelin components (3) (fig. S5), we assessed whether Taxol treatment alone could promote growth of injured CNS axons. To this end, we examined the effect of 4-week continuous Taxol treatment on axonal growth of the Raphe-spinal tract after dorsal hemisection (22, 23). Immunostaining revealed an increase by a factor of 5 in the number of serotonin [5-hydroxytryptamine (5-HT)]–positive fibers in the spinal cord caudal to the lesion in the Taxol-treated animals compared with vehicle (Fig. 4, C and D). The causal edge of the lesion was enriched in 5-HT–positive axons (Fig. 4, C and D). Axonal tips showed growth cones rather than retraction bulbs (fig. S6) and, frequently, out of the retraction bulbs a new process emanated (fig. S6). Additionally, we observed an ectopic distribution of 5-HT fibers coursing along the dorsal part of the cord in Taxol-treated animals (Fig. 4C). Thus, Taxol induces growth of 5-HT axons after dorsal hemisection.

We next examined whether Taxol treatment leads to functional recovery after moderate spinal cord contusion (24). Similarly to the dorsal hemisection injury, Taxol treatment increased the number of 5-HT–positive fibers caudal to the lesion 8 weeks after injury (Fig. 4E). We tested locomotor performance by analyzing paw placement using a grid walk (25). After 2 and 4 weeks, Taxol- and vehicle-treated animals performed
equally on the grid walk (Fig. 4F). However, while vehicle-treated animals did not show additional recovery, Taxol-treated animals improved further to a 5% misstep frequency after 6 and 8 weeks, resulting in improvement by a factor of 3.4 (Fig. 4F and movies S3 and S4). Thus, Taxol-induced functional recovery correlates with its axon growth–inducing effect.

Current attempts to elicit axonal regeneration in the injured spinal cord include interference with extrinsic growth inhibitory factors present in CNS myelin and scar tissue (18, 19, 26) and their various signaling pathways (27–30). Whereas these approaches aim to interfere with single inhibitory factors, we targeted the cytoskeleton, onto which growth inhibitory signaling pathways converge (28). Moreover, microtubules disassemble in injured CNS axons and, thereby, fail to provide protrusive force necessary for axon outgrowth (3, 4). Here, moderate stabilization of microtubules counteracted various cellular processes that prevent axon regeneration. Thus, Taxol has the potential to offer a multitargeted therapy for SCI.

**References and Notes**

1. X. Liu et al., PLoS Med. 2, e354 (2005).
2. S. Westermann, K. Weber, Nat. Rev. Mol. Cell Biol. 4, 938 (2003).
3. A. Erðük, F. Hellali, J. Enes, F. Bradke, J. Neurosci. 27, 9169 (2007).
4. H. Witte, D. Neukirchen, F. Bradke, J. Cell Biol. 180, 619 (2008).
5. N. Klapka, H. W. Müller, J. Neurotrauma 23, 422 (2006).
6. M. C. Shearer et al., Mol. Cell. Neurosci. 24, 913 (2003).
7. J. Silver, J. H. Miller, Nat. Rev. Neurosci. 5, 116 (2004).
8. B. E. Rovinsky, L. A. Cazeneuve, R. C. Donehower, J. Natl. Cancer Inst. 82, 1247 (1990).
9. S. Okada et al., Nat. Med. 12, 829 (2006).
10. C. Schachtrup et al., J. Neurosci. 30, 5843 (2010).
11. H. L. Moses, R. J. Coffey Jr., E. B. Leaf, R. M. Lyons, J. Kesi-K-Oja, J. Cell Physiol. Suppl. 133 (S5), 1 (1987).
12. D. Lindholm, E. Castron, R. Kleve, F. Zafra, H. Thoenen, J. Cell Biol. 117, 395 (1992).
13. J. Batut, M. Howell, C. S. Hill, Dev. Cell 12, 261 (2007).
14. C. Dong, Z. Li, R. Alvarez Jr., X. H. Feng, P. J. Goldschmidt-Clermont, Mol. Cell 5, 27 (2000).
15. S. Dunn et al., J. Cell Sci. 121, 1085 (2008).
16. L. C. Kaplan et al., Biophys. J. 99, 2143 (2010).
17. L. J. Jones, Y. Yamaguchi, W. B. Stallcup, M. H. Tuszynski, J. Neurosci. 22, 2792 (2002).
18. E. J. Bradbury et al., Nature 416, 636 (2002).
19. A. Hurtado, H. Podolin, M. Oudega, B. Grimpe, Brain 131, 2596 (2008).
20. T. L. Laabs et al., J. Neurosci. 27, 14494 (2007).
21. B. Viera et al., Curr. Biol. 19, 930 (2009).
22. J. Dill, H. Wang, F. Zhou, S. Li, J. Neurosci. 28, 8914 (2008).
23. D. D. Pearse et al., Nat. Med. 10, 610 (2004).
24. S. W. Schef, A. G. Rabchevsky, I. Fugaccia, J. A. Main, J. L. Lummier, J. Neurotrauma 20, 179 (2003).
25. D. L. Behrmann, J. C. Brensahm, S. M. Beattie, B. R. Shah, J. Neurotrauma 9, 197 (1992).
26. P. Freund et al., Nat. Med. 12, 790 (2006).
27. P. Dergham et al., J. Neurosci. 22, 6570 (2002).
28. G. Yi, Z. He, Nat. Rev. Neurosci. 7, 617 (2006).
29. S. Neumann, F. Bradke, M. Tessier-Lavigne, A. I. Basbaum, Neuron 34, 885 (2002).
30. J. Qiu et al., Neuron 34, 895 (2002).
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**Supporting Online Material**

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Materials and Methods

Figs. S1 to S7

Movies S1 to S4

References

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