Facilitating axon regeneration in the injured CNS by microtubules stabilization

Vetrivel Sengottuvel and Dietmar Fischer*

Department of Experimental Neurology; Heinrich-Heine University of Düsseldorf; Düsseldorf, Germany

Key words: Taxol, microtubules, axon, regeneration, CNTF, LIF, central nervous system, retina

Traumatic CNS injuries often cause permanent, devastating disabilities due to a lack of regeneration of damaged axons. Next to an insufficient intrinsic capability of CNS neurons to regrow axons, also inhibitory molecules that are associated with the CNS myelin and the glial scar contribute to the failure of axonal regeneration. Strategies targeting the inhibitory molecules, their receptors or downstream signaling pathways result in little improvement of regeneration in vivo. However, the combination of such approaches together with measures that increase the intrinsic growth potential of neurons reportedly lead to a significantly better outcome. In this mini-review we outline and discuss a novel therapeutic strategy facilitating axon regeneration by directly targeting microtubule dynamics in axonal growth cones and reducing the inhibitory scar formation at the injury site by the anti-cancer drug Taxol. Moreover, we portray the mechanisms underlying the beneficial effects of Taxol and its potential as an adjuvant drug to accomplish substantial regeneration and functional recovery after CNS injuries in vivo.

Damage of the optic nerve or spinal cord injury can result in devastating disabilities, such as irreversible blindness or paralysis. These disabilities are often due to an insufficient capacity of the central nervous system (CNS) to regenerate injured axons and are partially attributable to a destabilization of growth cones by molecules associated with the CNS white matter (e.g., Nogo, myelin associated glycoprotein and oligodendrocyte-myelin glycoprotein) and/or the glial scar (e.g., chondroitin sulfate proteoglycans, CSPGs).1,2 Exposure of injured axon tips to central myelin or CSPGs leads via specific receptors and activation of RhoA/ROCK-signaling to a depolymerisation of actin filaments in growth cones and reducing the inhibitory microtubules-actin interaction in the growth cone.12,13 Thus, myelin inhibitors and CSPGs indirectly compromise microtubules extension. In our recent article we directly targeted microtubules dynamics using the clinically established anti-cancer drug Paclitaxel (Taxol).14 Taxol can differentially affect the microtubule dynamics based on the concentrations at which it is applied. At higher concentrations, as typically used for cancer therapy, Taxol hyperstabilizes microtubules, thereby abrogates microtubule extension and inhibits mitotic spindle assembly, which is essential for cell division. However, at lower concentrations Taxol favors the polymerization of microtubules at the plus ends.15,16 Hence, we hypothesized that at low concentrations Taxol might enhance polymerization of microtubules in growth cones and thereby improve axon growth of mature CNS neurons. Indeed, axon outgrowth of mature RGCs was markedly increased on a growth permissive substrate in culture when Taxol was applied at a concentration of 1–3 nM, whereas at a concentration of 10 nM it had no effect. At a concentration of 50 nM the drug even reduced outgrowth compared to untreated controls. The beneficial effects of Taxol were compromised by nocodazole a compound that increases the catastrophe rate of microtubules, suggesting that Taxol indeed enhanced neurite extension directly by microtubules stabilization. The effects of Taxol on neurite growth were further increased by the coadministration of ciliary neurotrophic factor (CNTF), implying that both molecules acted synergistically through different mechanisms. CNTF mediates its effects via the activation of various signaling pathways in the cell body of neurons.17,18 In contrast, Taxol treatment stabilized growth cones and completely overcame the regeneration in the optic nerve.8,9 However, these studies and others have also shown that overcoming inhibitory signaling alone results only in little regeneration, since mature central neurons, contrary to embryonic or postnatal neurons, possess only a weak intrinsic capacity to regrow axons even in the absence of inhibitors. Therefore, combinatorial treatments aiming to activate the intrinsic growth ability and measures to overcome inhibitory signaling result in significant better axon regeneration than each treatment alone.8,11

Microtubules Stabilization Facilitates Axonal Growth and Desensitizes Growth Cones Towards Inhibitory Molecules In Vitro

Microtubules polymerization is essential for axonal growth and is modulated by microtubules-actin interaction in the growth cone.12,13 Thus, myelin inhibitors and CSPGs indirectly compromise microtubules extension. In our recent article we directly targeted microtubules dynamics using the clinically established anti-cancer drug Paclitaxel (Taxol).14 Taxol can differentially affect the microtubule dynamics based on the concentrations at which it is applied. At higher concentrations, as typically used for cancer therapy, Taxol hyperstabilizes microtubules, thereby abrogates microtubule extension and inhibits mitotic spindle assembly, which is essential for cell division. However, at lower concentrations Taxol favors the polymerization of microtubules at the plus ends.15,16 Hence, we hypothesized that at low concentrations Taxol might enhance polymerization of microtubules in growth cones and thereby improve axon growth of mature CNS neurons. Indeed, axon outgrowth of mature RGCs was markedly increased on a growth permissive substrate in culture when Taxol was applied at a concentration of 1–3 nM, whereas at a concentration of 10 nM it had no effect. At a concentration of 50 nM the drug even reduced outgrowth compared to untreated controls. The beneficial effects of Taxol were compromised by nocodazole a compound that increases the catastrophe rate of microtubules, suggesting that Taxol indeed enhanced neurite extension directly by microtubules stabilization. The effects of Taxol on neurite growth were further increased by the coadministration of ciliary neurotrophic factor (CNTF), implying that both molecules acted synergistically through different mechanisms. CNTF mediates its effects via the activation of various signaling pathways in the cell body of neurons.17,18 In contrast, Taxol treatment stabilized growth cones and completely overcame the
neurite outgrowth inhibition by myelin without affecting the myelin-induced activation of RhoA. This disinhibitory effect of Taxol was also found for inhibitory CSPGs, implying that it is not restricted to specific receptors or pathways, but rather mediated by microtubules polymerization. One possible explanation for the disinhibitory effect of Taxol might be the disentanglement of the coupled interaction of actin and microtubules in the growth cone. This makes the microtubule polymerization at the plus ends independent of the state of actin polymerization and thereby stabilizes the overall cytoskeleton structure in the growth cone, including the microtubules in the filopodial extensions.14,19 Thus, in a defined concentration range Taxol exerts two beneficial effects that are relevant for axon regeneration: it promotes axon extension and decreases the sensitivity of growth cones towards inhibitory molecules.

Local Application of Taxol Facilitates Axon Regeneration In Vivo

The optic nerve is a widely used model system to study the regenerative failure of CNS axons. In vivo mature RGCs can be transformed into a regenerative state by lens injury or intravitreal application of zymosan or the toll-like receptor 2 agonist PamCys.20-24 The transformation of RGCs into a regenerative state is associated with a dramatic change in gene expression, which helps RGCs to survive optic nerve injury and also extend axons beyond the optic nerve injury site.8 Astrocyte-derived CNTF and leukemia inhibitory factor (LIF) have been identified as the essential key factors mediating both the neuroprotective and axon growth-promoting effects after lens injury.17,25,26 Nevertheless, the regeneration of stimulated RGCs is still limited by the inhibitory environment of the glial scar and myelin.

Our recent paper demonstrates that locally applied Taxol, although not affecting the regenerative state or survival of RGCs, markedly augmented initial growth of axons beyond the lesion site of the optic nerve.14 These effects may be due to its disinhibitory and axon growth-promoting effects observed in cell culture. When evaluated 14 days after injury, local Taxol application only moderately improved axonal regeneration in the optic nerve. However, regeneration was dramatically enhanced when Taxol treatment was combined with a stimulation of the regenerative state of RGCs by lens injury. The regeneration of axons 1 mm beyond the lesion site was 15 times higher with the combinatorial treatment compared to lens injury alone. Notably, although Taxol exerted beneficial effects at a broad concentration range in vivo, its effects were, as observed in culture, concentration dependent with strongest effects measured at low concentrations. This finding may be relevant in view of a potential therapeutic use for nerve repair in humans, since it may lower the risk for known adverse side effects associated with the application of much higher dosages of the drug in cancer therapy. In contrast to locally applied Taxol at the injury site, intravitreal injections of Taxol did not facilitate axon regeneration, suggesting that the growth promoting effects were due to an interaction of the drug with the microtubules in growth cones and, as discussed below, to the delayed glial scar formation.

In accordance to our results, another concurrently published study has reported that the continuous application of low concentrations of Taxol into the lesion site of the spinal cord combined with an activation of the regenerative state of dorsal root ganglion neurons through a peripheral conditioning lesion improved axon regeneration and functional recovery.27 These data demonstrate that microtubules stabilization by Taxol is a suitable approach to successfully facilitate axon regeneration in different areas of the CNS.

Taxol Treatment Delays Glial Scar Formation

Glial scar formation is one of the major impediments that hinders axon regeneration after CNS injury. The events that occur around the lesion site include the proliferation of astrocytes, which leads to astrogliosis hypertrophy and expression of inhibitory molecules such as CSPGs.2 In addition, the activation of microgliosis and recruitment of peripheral macrophages from the blood stream are also involved in glial scar formation. The latter ones have been recently proposed to function as another significant barrier for axon regeneration.28,29 Since Taxol is an anti-proliferative drug and also known to affect cell migration,30,31 it was plausible that locally applied Taxol might also influence the glial scar formation. Indeed, even at low concentrations Taxol transiently delayed the proliferation of astrocytes and the infiltration of macrophages around the lesion site after optic nerve injury. Notably, Taxol suppressed the expression of inhibitory CSPGs in the injured optic nerve a finding that was also reported in the injured spinal cord.14,27 One explanation for the CSPG suppression could be an inhibition of transforming growth factor beta (TGFβ) signaling by Taxol, which is necessary for the inhibitory scar formation.32,33 Previous reports proposed that microtubules destabilization modulates TGFβ signaling by favoring phosphorylation of Smads and translocation of the microtubule bound-transcription factors from the cytoplasm to the nucleus.34 Thus, microtubule stabilization by Taxol enhances the binding of Smad to microtubules, thereby diminishing the translocation of Smads to the nucleus and abrogating TGFβ signaling.35 Another explanation could be that the expression of TGFβ per se is reduced by the Taxol treatment, since Taxol delays the proliferation and migration of glial cells and macrophages, which are known sources of TGFβ at the lesion site. Regardless of the underlying mechanism, the delay in glial scar formation and reduction of macrophage invasion by Taxol treatment may have also contributed to the augmented regeneration observed after optic nerve injury.

Perspectives

Current reports demonstrate that Taxol, when locally applied at the injury site fosters axon regeneration by microtubules stabilization in the axonal growth cone and delays the inhibitory glial scar formation.14,27 Moreover, Taxol exerts its beneficial effects on axon regeneration at low concentrations, which reduces the risk of potential adverse side effects. Taxol is also a clinically approved drug for humans which makes this compound a promising candidate as an adjuvant drug for the treatment of patients suffering
from traumatic CNS injuries or stroke. Additional optimization of modalities of drug application may further improve the beneficial outcome on axon regeneration and be necessary for a potential application in humans. In fact, a previous study has reported that the systemic application of Taxol supports functional recovery of rats after spinal cord injury, although it is not clear whether these effects were the result of improved axonal regeneration or caused by other mechanisms. Nevertheless, very recently published results from both, optic nerve and spinal cord injury models lend encouragement to the possibility that microtubule-stabilizing compounds such as Taxol may be suitable adjuvant drugs for the treatment of CNS injuries particularly when combined with interventions stimulating the intrinsic regenerative state of neurons.

References

1. Yu G, He Z. Glial inhibition of CNS axon regeneration. Nat Rev Neurosci 2006; 7:617-27.
2. Silver J, Miller JH. Regeneration beyond the glial scar. Nat Rev Neurosci 2004; 5:146-56.
3. Mukhopadhyay G, Doherty P, Walsh FS, Crocker PR, Filbin MT. A novel role for myelin-associated glycoprotein as an inhibitor of axonal regeneration. Neuron 1994; 13:757-67.
4. GrandPrT, Nakamura F, Vartanian T, Strittmatter SM. Identification of the Nogo inhibitor of axon regeneration as a Reticulon protein. Nature 2000; 403:439-44.
5. Piniha R, Moore SE, Vinson M, Blake S, Morrow R, Christie G, et al. Inhibitor of neurite outgrowth in humans. Nature 2000; 403:883-4.
6. Wang KC, Kopitova V, Kim JA, Sivasankaran R, Goo Y, Neve RL, et al. Oligodendrocyte-myelin glycoprotein is a Nogo receptor ligand that inhibits neurite outgrowth. Nature 2002; 417:941-4.
7. Arwal JK, Pinkenon-Gousse J, Syken J, Stawicki S, Wu Y, Shatz C, et al. PcdB is a functional receptor for myelin inhibitors of axonal regeneration. Science 2008; 322:967-70.
8. Fischer D, Petkova V, Thanos S, Benowitz LI. Switching mature retinal ganglion cells to a robust growth state in vivo: gene expression and synergy with Rhoa inactivation. J Neurosci 2004; 24:8722-31.
9. Fischer D, He Z, Benowitz LI. Counteracting the Nogo receptor enhances optic nerve regeneration if retinal ganglion cells are in an active growth state. J Neurosci 2004; 24:1646-51.
10. Leaver SG, Cui Q, Plant GW, Arulpragasam A, Huish S, Verhaegen J, et al. AAV-mediated expression of CNTF promotes long-term survival and regeneration of adult rat retinal ganglion cells. Gene Ther 2006; 13:1328-41.
11. Steinmetz MP, Horn KP, Tom VJ, Miller JH, Busch SA, Nair D, et al. Chronic enhancement of the intrinsic growth capacity of sensory neurons combined with the degradation of inhibitory proteoglycans allows functional regeneration of sensory axons through the dorsal root entry zone in the mammalian peripheral cord. J Neurosci 2005; 25:8066-76.
12. Dent EW, Kalil K. Axon branching requires interactions between dynamic microtubules and actin filaments. J Neurosci 2001; 21:9757-69.
13. Tanaka E, Ho T, Kirschner MW. The role of microtubule dynamics in growth cone motility and axonal growth. J Cell Biol 1995; 128:139-55.
14. Sengotuvel V, Leibinger M, Pfeiffer M, Andreadaki A, Fischer D. Taxol facilitates axon regeneration in the mature CNS. J Neurosci 2011; 31:2688-99.
15. Derry WB, Wilson L, Jordan MA. Substoichiometric binding of taxol suppresses microtubule dynamics. Biochemistry 1995; 34:2203-11.
16. Derry WB, Wilson L, Khan IA, Luduena RF, Jordan MA. Taxol differentially modulates the dynamics of microtubules assembled from unpurified and purified beta-tubulin isotypes. Biochemistry 1997; 36:3554-62.
17. Müller A, Hauk TG, Leibinger M, Marienhoff R, Fischer D. Enzyme-linked CNTF stimulates axon regeneration of retinal ganglion cells partially via endogenous CNTF. Mol Cell Neurosci 2009; 41:233-46.
18. Park K, Luo JM, Huish S, Harvey AR, Cui Q. Cellular mechanisms associated with spontaneous and cilary neurotrophic factor-CAMP-induced survival and axonal regeneration of adult retinal ganglion cells. J Neurosci 2004; 24:10806-15.
19. Geraldo S, Gordon-Weeks PR. Cytoskeletal dynamics in growth-cone steering. J Cell Biol 2009; 122:595-604.
20. Fischer D, Pavlidis M, Thanos S. Catastrophic lens injury prevents traumatic ganglion cell death and promotes axonal regeneration both in vivo and in culture. Invest Ophthalmo Vis Sci 2000; 41:3943-54.
21. Hauk TG, Leibinger M, Müller A, Andreadaki A, Knüppling U, Fischer D. Intravitreal application of the Toll-like receptor 2 agonist Pam3CSK4 stimulates axon regeneration in the mature optic nerve. Invest Ophthalmo Vis Sci 2009; 51:459-64.
22. Hauk TG, Müller A, Lee J, Schwendener R, Fischer D. Neuroprotective and axon growth promoting effects of intracocular inflammation do not depend on eno- modulin or the presence of large numbers of activated macrophages. Exp Neurol 2008; 209:469-82.
23. Levinson S, Yim Y, Nguyen J, Irwin N, Benowitz LI. Lens injury stimulates axon regeneration in the mature rat optic nerve. J Neurosci 2000; 20:4615-26.
24. Lovett-Barron A, Berry M, Logan A. Lens injury stimulates adult retinal ganglion cell axon regeneration via both macrophage- and lens-derived factors. Eur J Neurosci 2005; 21:2029-34.
25. Leibinger M, Müller A, Andreadaki A, Hauk TG, Kirsch M, Fischer D. Neuroprotective and axon growth-promoting effects following inflammatory stimulation on mature retinal ganglion cells in mice depend on ciliary neurotrophic factor and leukemia inhibitory factor. J Neurosci 2009; 29:14334-41.
26. Muller A, Hauk TG, Fischer D. Astrocyte-derived CNTF switches mature RG#Cs to a regenerative state following inflammatory stimulation. Brain 2007; 130:3308-20.
27. Hellf R, Hurtado A, Ruschel J, Flynn KC, Laskowski CJ, Umlauf M, et al. Microtubule stabilization reduces scarring and causes axon regeneration after spinal cord injury. Science 2011; 331:928-31.
28. Busch SA, Horn KP, Silver DJ, Silver J. Overcoming macrophage-mediated axonal dieback following CNS injury. J Neurosci 2009; 29:9607-76.
29. Maranha RC, Tavares ER, Padoveze AF, Valduga CJ, Rodrigues DG, Pereira MD. Paclitaxel associated with cholesterol-rich nanoemulsions promotes athersclerosis regression in the rabbit. Atherosclerosis 2008; 197:959-66.
30. Winkinchen J, Schöber W, Schatt N, Kehlback R, Wiesebe A, Tepe G, et al. The effects of paclitaxel on the three phases of restenosis: smooth muscle cell proliferation, migration and matrix formation: in an in vitro study. Invest Radiol 2004; 39:565-71.
31. Schachturc G, Rocky JK, Helmich MJ, Vagana E, Balanakis DK, Degen JL, et al. Fibrinogen triggers astrocyte scar formation by promoting the availability of active TGFbeta after vascular damage. J Neurosci 2010; 30:5843-54.
32. Wang Y, Moges H, Bharucha Y, Symes A, Smad3 null mice display more rapid wound closure and reduced scar formation after a stab wound to the cerebral cortex. Exp Neurol 2007; 203:168-84.
33. Dong C, Li Z, Alvarez R Jr, Feng XH, Goldsmith-Clemont PJ. Microtubule binding to Smads may regulate TGF beta activity. Mol Cell 2000; 5:27-34.
34. Perez-Espejo MA, Haghighi SS, Adelstein EH, Maden M. The effects of taxol, methylprednisolone and 4-aminopyridine in compressive spinal cord injury: a qualitative experimental study. Surg Neurol 1996; 46:350-7.