Review

Efficacy of epothilones in central nervous system trauma treatment: what has age got to do with it?

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Abstract

Central nervous system injury, specifically traumatic brain and spinal cord injury, can have significant long lasting effects. There are no comprehensive treatments to combat the injury and sequalae of events that occurring following a central nervous system trauma. Herein we discuss the potential for the epothilone family of microtubule stabilizing agents to improve outcomes following experimentally induced trauma. These drugs, which are able to cross the blood-brain barrier, may hold great promise for the treatment of central nervous system trauma and the current literature presents the extensive range of beneficial effects these drugs may have following trauma in animal models. Importantly, the effect of the epothilones can vary and our most recent contributions to this field indicate that the efficacy of epothilones following traumatic brain injury is dependent upon the age of the animals. Therefore, we present a case for a greater emphasis to be placed upon age when using an intervention aimed at neural regeneration and highlight the importance of tailoring the therapeutic regime in the clinic to the age of the patient to promote improved patient outcomes.

Key Words: aging; epothilones; glial; microtubule stablization; neuron; neuronal regeneration; spinal cord injury; traumatic brain injury

Traumatic brain and spinal cord injuries continue to be a leading cause of death and disability in developed nations (Stocchetti and Zanier, 2016). This trauma can result in primary damage and complex secondary pathologies, which can cause prolonged or life-long motor and/or cognitive impairments. There are currently no available therapeutics that are able to prevent, minimize or reverse the deficits that develop following central nervous system (CNS) trauma. Effective therapeutic strategies are not being discovered fast enough and many promising drugs in the preclinical setting are not proving effective in clinical trial. A recurrent theme over the last decade has been to focus on why so many promising new therapeutics are failing to jump the gap from bench to bedside. One possible limitation to preclinical trials, that may not be accounted for appropriately, is the effect of age on the heterogeneity of symptoms and functional deficits following a CNS insult (Sun et al., 2020). Here we examine the evidence for a promising therapy for treating CNS trauma, the epothilones, which have potent neuronal and non-neuronal cell actions principally through microtubule stabilization, and propose that the efficacy of these drugs and likelihood of success in translating to the clinic may depend upon stratifying for age at insult.

A consequence of CNS trauma is neuronal cell loss and axonal injury, and the activation and reaction of glial populations (Spain et al., 2010; Wang and Ma, 2010; Greer et al., 2011; Hassannejad et al., 2019). Following injury microglia, oligodendroglial precursors, meningeal cells and astrocytes concentrate within the injury site. These cells can have both favorable and detrimental effects on the neuronal regenerative response (Ng and Lee, 2019). The formation of the glial scar presents a chemical and physical barrier to stop the spread of the injury, while conversely inhibiting regeneration of disconnected axons (Fawcett and Asher, 1999). Axons are particularly vulnerable to structural injury due to their relative long length and size. Injury-induced axonal degeneration can have devastating and far-reaching effects. In severe trauma axons are severed, and this disconnection can lead to a starvation of the post synaptic connection and disruption and retraction of the proximal neuron (Blennow et al., 2016). In mild to moderate traumatic brain injury, axons are often subtly disrupted at the time of injury, with immediate changes including mechanically induced alterations in permeability, deregulation of ionic homeostasis and compression of the cytoskeleton that can finally lead to disconnection (Smith et al., 2013). Hence, regardless of the initial injury severity cytoskeletal misalignment or loss and the accumulation or compaction of cytoskeletal components is a common event. The microtubule cytoskeleton has been shown to be particularly disrupted following structural injury: Microtubule alterations after injury include microtubule disruption and detachment with associated defects in axonal transport (Smith et al., 1999; Tang-Schomer et al., 2010) and the loss of microtubule-associated proteins such as Tau and microtubule-associated protein 2 (Farkas and Povlishock, 2007; Bradke et al., 2012; Smith et al., 2013).

Systematic database searches of PubMed and Web of Science were performed to identify valid peer reviewed studies with no limitations on year of publication. Keyword terms used focused on CNS aging, CNS trauma, traumatic brain injury,

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microtubule stabilisation, epothilones and taxanes, axonal regeneration and neuronal regeneration. We used a narrow selection criteria for this review, and other variables should be considered to gain a greater understanding on age dependant CNS trauma and therapeutic targeting.

Critical to the treatment of CNS trauma is the restoration of neuronal and glial function. Microtubule stabilizing drugs, including Food and Drug Administration approved taxanes and the epothilones, hold the potential to effect both neuronal and glial populations. Taxanes and the epothilones are already used at relatively high doses in the treatment of various cancers where, due to their hyper-stabilization of microtubules, they act to arrest cell division and slow or prevent cancer cell growth (Goodin et al., 2004; Kolman, 2004). However, it is their action at low doses that holds particular promise for treating CNS injury. At low concentrations these same drugs can protect against microtubule depolarization and dissolution, and encourage polarization and structural stability (Brunden et al., 2010; Hellal et al., 2011; Sengottuvel and Fischer, 2011; Baas and Ahmad, 2013). This quality could be particularly beneficial for protecting axons following structural injury. Indeed, paclitaxel, one of the taxanes, has been shown to improve outcomes following models of nerve injury by promoting axonal elongation and regeneration and also reducing glial scar formation (Hellal et al., 2011; Sengottuvel et al., 2011). However, paclitaxel has poor blood-brain barrier permeability, making it a sub-optimal candidate for CNS delivery. The epothilones, a group of microtubule stabilizing compounds found naturally in the myxobacterium S*orangium cellulosum* (Bollag et al., 1995), offer an attractive alternative solution. Numerous epothilones have now been identified, including Epothilone B (EpoB) and Epothlione D (EpoD), which share a mode of action similar to paclitaxel for the binding site on β-tubulin (Brunden et al., 2011). Due to their increased water solubility, the epothilones readily crosses the blood-brain barrier and can be retained within the CNS for several days (Andrieux et al., 2006; Cortes and Baselga, 2007; Brunden et al., 2012).

An exciting publication in 2015 cemented the epothilones as a promising therapeutic intervention following CNS injury. Using EpoB, administered following spinal cord injury, the authors convincingly demonstrated EpoB therapy led to increased axon outgrowth, reduced scarring and improved functional recovery in female rats (Ruschel et al., 2015). The axonal outgrowth was improved through increased microtubule polymerization, and consequently microtubule protrusion into regenerating axons. This work has been supported with evidence for similar efficacy of EpoD (Ruschel and Bradke, 2018; Sandner et al., 2018), its effect on other cell types (Zhao et al., 2017) and other *in vivo* experimental models of axonal injury such as spinal and corneal nerve injury (Li and Wu, 2017; Wang et al., 2018). A positive effect has also been reported in EpoB administered following intracerebral hemorrhage, in which it restored the integrity of the nigrostriatal pathway neuronal circuit and improve fine motor functional recovery after injury in mice (Yang et al., 2018). These findings follow similar promising results seen in neurodegenerative disease; EpoD treatment resulted in improved outcomes in *in vivo* models of Parkinson's disease (Cartelli et al., 2013), tauopathy and Alzheimer's disease (Brunden et al., 2010; Zhang et al., 2012), and schizophrenia (Andrieux et al., 2006; Fournet et al., 2012). However, a collection of studies also reports subtle or adverse effects of epothilones in some neurodegenerative models (Mao et al., 2017; Clark et al., 2018). This discrepancy in findings raises the question of why does the effect of epothilone administration vary across different experimental investigations?

Given Traumatic brain and spinal cord injuries can be acquired at any stage of life, age may play a critical role in outcomes following CNS trauma (Sun et al., 2020). Based on our recent studies, we propose that age is not only an important factor in dictating outcomes following CNS trauma, but also plays a role in determining the efficacy of microtubule stabilizers, such as EpoD, as a post injury intervention. When studying the effect of EpoD *in vitro*, we found that the vulnerability of cortical neurons to EpoD increased as the age of the neuron increased (Clark et al., 2020). These findings are in line with those of Jang et al. (2016), who found an age-related contribution in response to EpoB treatment in both cortical and dorsal root ganglion cells *in vitro*. Moreover, using an *in vivo* model of trauma (lateral fluid percussion brain injury) we have shown that a single peripherally administered dose of EpoD targeted central neurons, specifically increasing the density of mushroom spines on layer 5 cortical pyramidal neurons, with an absence of astroglial effects (Chuckowree et al., 2018). To add further evidence to these findings, we have previously investigated the protective effect of EpoD in a mouse model of amyotrophic lateral sclerosis (Clark et al., 2018). This therapy led to worse survival outcomes, greater functional deficits and an increase in microglial and astroglial activation at end stage– approximately 6 months old. However, this treatment initially prevented motor neuron loss and axonal degeneration at 2 months of age, suggesting that there may be a differing effect as either the disease progressed or the mouse aged. Together, these studies suggest that age may differentially impact the efficacy of EpoD on neuronal and glial populations.

To directly address the effect of age on EpoD efficacy, we exposed young, adult and aged mice to an *in vivo* brain injury (lateral fluid percussion brain injury). We found that the degree of axonal degeneration as well as astrogliosis and microglial activation were age dependent (Zhu et al., 2020). Critically, we determined that EpoD administration had very different effects in young (1.5 months) *versus* adult (3 months) mice. In young mice, EpoD administration trended to confer protection from axonal degeneration. However, when the same dose was given to adult mice, EpoD had a detrimental effect – axonal degeneration in the internal capsule white matter tract was significantly increased. Collectively, these studies provide compelling evidence that age is an important contributor to outcomes following therapeutic intervention with epothilone derivatives.

How exactly age is affecting epothilone efficacy, is not clear. Within the neuron, microtubules contribute to a range of neuronal functions including neurite outgrowth, neuronal polarity, axonal transport and regulating gene expression and signaling pathways (Dubey et al., 2015). Interestingly microtubule loss has not only been seen in Alzheimer's disease but can also be present in cases of normal aging (Cash et al., 2003). How microtubule function changes over time and how microtubule stabilizing agents bind remains to be defined. Furthermore, the mechanism of the effect of age is multifactorial; likely to involve glial cells (Ritzel et al., 2019; Sun et al., 2019; Webster et al., 2019) as well as neurons (Sun et al., 2019). EpoD has been shown to reduce the glial scar and affect both fibroblasts and immune cells such as microglia following spinal cord injury (Ruschel et al., 2015; Mao et al., 2017). The function of microglia changes throughout the lifespan, and these alterations can exacerbate the injury response in an aged system (Morganti-Kossmann et al., 2019; Sun et al., 2020). This altered microglial response can be correlated to a decrease in functional recovery following injury, demonstrating the impact of the aging system on outcomes (Ritzel et al., 2019; Sun et al., 2019). It is important to also note that the heterogeneity of the response to epothilone treatment may not be limited to ageing. The response of the CNS to trauma can be sex-dependent (Inampudi et al., 2020) – how this affects therapeutic intervention outcomes is largely unexplored.

In conclusion, mounting evidence in both trauma and neurodegenerative disease models highlights the need for

future studies determining how the efficacy of therapeutic microtubule stabilization is altered across the lifespan. Understanding how age contributes to therapeutic intervention following trauma could pave the way to providing a more tailored therapeutic regime in the clinic that is specific to the age of the patient.

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References

Andrieux A, Salin P, Schweitzer A, Bégou M, Pachoud B, Brun P, Gory-Fauré S, Kujala P, Suaud-Chagny M-F, Höfle G (2006) Microtubule stabilizer ameliorates synaptic function and behavior in a mouse model for schizophrenia. Biol Psychiatry 60:1224-1230.

Baas PW, Ahmad FJ (2013) Beyond taxol: microtubule-based treatment of disease and injury of the nervous system. Brain 136:2937-2951.

Blennow K, Brody DL, Kochanek PM, Levin H, McKee A, Ribbers GM, Yaffe K, Zetterberg H (2016) Traumatic brain injuries. Nat Rev Dis Primers 2:16084.

Bollag DM, McQueney PA, Zhu J, Hensens O, Koupal L, Liesch J, Goetz M, Lazarides E, Woods CM (1995) Epothilones, a new class of microtubule-stabilizing agents with a txol-like mechanism of action. Cancer Res 55:2325-2333.

Bradke F, Fawcett JW, Spira ME (2012) Assembly of a new growth cone after axotomy: the precursor to axon regeneration. Nat Rev Neurosci 13:183-193.

Brunden KR, Ballatore C, Lee VMY, Smith AB, Trojanowski JQ (2012) Brain-penetrant microtubule-stabilizing compounds as potential therapeutic agents for tauopathies. Biochem Soc Trans 40:661-666.

Brunden KR, Zhang B, Carroll J, Yao Y, Potuzak JS, Hogan AML, Iba M, James MJ, Xie SX, Ballatore C (2010) Epothilone D improves microtubule density, axonal integrity, and cognition in a transgenic mouse model of tauopathy. J Neurosci 30:13861-13866.

Brunden KR, Yao Y, Potuzak JS, Ferrer NI, Ballatore C, James MJ, Hogan AML, Trojanowski JQ, Smith AB, Lee VMY (2011) The characterization of microtubule-stabilizing drugs as possible therapeutic agents for Alzheimer's disease and related tauopathies. Pharmacol Res 63:341-351.

Cartelli D, Casagrande F, Busceti CL, Bucci D, Molinaro G, Traficante A, Passarella D, Giavini E, Pezzoli G, Battaglia G (2013) Microtubule alterations occur early in experimental parkinsonism and the microtubule stabilizer epothilone D is neuroprotective. Sci Rep 3:1837.

Cash AD, Aliev G, Siedlak SL, Nunomura A, Fujioka H, Zhu X, Raina AK, Vinters HV, Tabaton M, Johnson AB, Paula-Barbosa M, Avíla J, Jones PK, Castellani RJ, Smith MA, Perry G (2003) Microtubule reduction in Alzheimer's disease and aging is independent of τ filament formation. Am J Pathol 162:1623-1627.

Chuckowree JA, Zhu Z, Brizuela M, Lee KM, Blizzard CA, Dickson TC (2018) The microtubule-modulating drug epothilone D alters dendritic spine morphology in a mouse model of mild traumatic brain injury. Front Cell Neurosci 12:223.

Clark JA, Blizzard CA, Breslin MC, Yeaman EJ, Lee KM, Chuckowree JA, Dickson TC (2018) Epothilone D accelerates disease progression in the SOD1(G93A) mouse model of amyotrophic lateral sclerosis. Neuropathol Appl Neurobiol 44:590-605.

Clark JA, Chuckowree JA, Dyer MS, Dickson TC, Blizzard CA (2020) Epothilone D alters normal growth, viability and microtubule dependent intracellular functions of cortical neurons in vitro. Sci Rep 10:918.

Cortes J, Baselga J (2007) Targeting the microtubules in breast cancer beyond taxanes: the epothilones. Oncologist 12:271-280.

Dubey J, Ratnakaran N, Koushika S (2015) Neurodegeneration and microtubule dynamics: death by a thousand cuts. Front Cell Neurosci 9:343.

Farkas O, Povlishock JT (2007) Cellular and subcellular change evoked by diffuse traumatic brain injury: a complex web of change extending far beyond focal damage. Prog Brain Res 161:43-59.

Fawcett JW, Asher RA (1999) The glial scar and central nervous system repair. Brain Res Bull 49:377-391.

Fournet V, Lavilléon G, Schweitzer A, Giros B, Andrieux A, Martres MP (2012) Both chronic treatments by epothilone D and fluoxetine increase the short-term memory and differentially alter the mood status of STOP/MAP6 KO mice. J Neurochem 123:982-996.

Goodin S, Kane MP, Rubin EH (2004) Epothilones: mechanism of action and biologic activity. J Clin Oncol 22:2015-2025.

Greer JE, McGinn MJ, Povlishock JT (2011) Diffuse traumatic axonal injury in the mouse induces atrophy, c-Jun activation and axonal outgrowth in the axotomized neuronal population. J Neurosci 31:5089-5105.

Hassannejad Z, Yousefifard M, Azizi Y, Zadegan SA, Sajadi K, Sharif-Alhoseini M, Shakouri-Motlagh A, Mokhatab M, Rezvan M, Shokraneh F, Hosseini M, Vaccaro AR, Harrop JS, Rahimi-Movaghar V (2019) Axonal degeneration and demyelination following traumatic spinal cord injury: A systematic review and meta-analysis. J Chem Neuroanat 97:9-22.

Hellal F, Hurtado A, Ruschel J, Flynn KC, Laskowski CJ, Umlauf M, Kapitein LC, Strikis D, Lemmon V, Bixby J (2011) Microtubule stabilization reduces scarring and causes axon regeneration after spinal cord injury. Science 331:928-931.

Jang EH, Sim A, Im SK, Hur EM (2016) Effects of microtubule stabilization by epothilone B depend on the type and age of neurons. Neural Plast 2016:5056418.

Kolman A (2004) Epothilone D (Kosan/Roche). Curr Opin Investig Drugs 5:657-667.

Li H, Wu W (2017) Microtubule stabilization promoted axonal regeneration and functional recovery after spinal root avulsion. Eur J Neurosci 46:1650-1662.

Mao L, Gao W, Chen S, Song Y, Song C, Zhou Z, Zhao H, Zhou K, Wang W, Zhu K, Liu C, Mei X (2017) Epothilone B impairs functional recovery after spinal cord injury by increasing secretion of macrophage colony-stimulating factor. Cell Death Dis 8:e3162.

Morganti-Kossmann MC, Semple BD, Hellewell SC, Bye N, Ziebell JM (2019) The complexity of neuroinflammation consequent to traumatic brain injury: from research evidence to potential treatments. Acta Neuropathol 137:731-755.

Ng SY, Lee AYW (2019) Traumatic brain injuries: pathophysiology and potential therapeutic targets. Front Cell Neurosci 13:528.

Ritzel RM, Doran SJ, Glaser EP, Meadows VE, Faden AI, Stoica BA, Loane DJ (2019) Old age increases microglial senescence, exacerbates secondary neuroinflammation, and worsens neurological outcomes after acute traumatic brain injury in mice. Neurobiol Aging 77:194-206.

Ruschel J, Bradke F (2018) Systemic administration of epothilone D improves functional recovery of walking after rat spinal cord contusion injury. Exp Neurol 306:243-249.

Ruschel J, Hellal F, Flynn KC, Dupraz S, Elliott DA, Tedeschi A, Bates M, Sliwinski C, Brook G, Dobrindt K, Peitz M, Brustle O, Norenberg MD, Blesch A, Weidner N, Bunge MB, Bixby JL, Bradke F (2015) Axonal regeneration. Systemic administration of epothilone B promotes axon regeneration after spinal cord injury. Science 348:347-352.

Sandner B, Puttagunta R, Motsch M, Bradke F, Ruschel J, Blesch A, Weidner N (2018) Systemic epothilone D improves hindlimb function after spinal cord contusion injury in rats. Exp Neurol 306:250-259.

Sengottuvel V, Fischer D (2011) Facilitating axon regeneration in the injured CNS by microtubules stabilization. Commun Integr Biol 4:391-393.

Sengottuvel V, Leibinger M, Pfreimer M, Andreadaki A, Fischer D (2011) Taxol facilitates axon regeneration in the mature CNS. J Neurosci 31:2688-2699.

Smith DH, Hicks R, Povlishock JT (2013) Therapy development for diffuse axonal injury. J Neurotrauma 30:307-323.

Smith DH, Wolf JA, Lusardi TA, Lee VMY, Meaney DF (1999) High tolerance and delayed elastic response of cultured axons to dynamic stretch injury. J Neurosci 19:4263-4269.

Spain A, Daumas S, Lifshitz J, Rhodes J, Andrews PJ, Horsburgh K, Fowler JH (2010) Mild fluid percussion injury in mice produces evolving selective axonal pathology and cognitive deficits relevant to human brain injury. J Neurotrauma 27:1429-1438.

Stocchetti N, Zanier ER (2016) Chronic impact of traumatic brain injury on outcome and quality of life: a narrative review. Crit Care 20:148.

Sun M, Brady RD, Casillas-Espinosa PM, Wright DK, Semple BD, Kim HA, Mychasiuk R, Sobey CG, O'Brien TJ, Vinh A, McDonald SJ, Shultz SR (2019) Aged rats have an altered immune response and worse outcomes after traumatic brain injury. Brain Behav Immun 80:536-550.

Sun M, McDonald SJ, Brady RD, Collins-Praino L, Yamakawa GR, Monif M, O'Brien TJ, Cloud GC, Sobey CG, Mychasiuk R, Loane DJ, Shultz SR (2020) The need to incorporate aged animals into the preclinical modeling of neurological conditions. Neurosci Biobehav Rev 109:114-128.

Tang-Schomer MD, Patel AR, Baas PW, Smith DH (2010) Mechanical breaking of microtubules in axons during dynamic stretch injury underlies delayed elasticity, microtubule disassembly, and axon degeneration. FASEB J 24:1401-1410.

Wang H, Xiao C, Dong D, Lin C, Xue Y, Liu J, Wu M, He J, Fu T, Pan H, Jiao X, Lu D, Li Z (2018) Epothilone B speeds corneal nerve regrowth and functional recovery through microtubule stabilization and increased nerve beading. Sci Rep 8:2647.

Wang HC, Ma YB (2010) Experimental models of traumatic axonal injury. J Clin Neurosci 17:157-162.

Webster KM, Sun M, Crack PJ, O'Brien TJ, Shultz SR, Semple BD (2019) Age-dependent release of high-mobility group box protein-1 and cellular neuroinflammation after traumatic brain injury in mice. J Comp Neurol 527:1102-1117.

Yang Y, Zhang X, Ge H, Liu W, Sun E, Ma Y, Zhao H, Li R, Chen W, Yuan J, Chen Q, Chen Y, Liu X, Zhang JH, Hu R, Fan X, Feng H (2018) Epothilone B benefits nigrostriatal pathway recovery by promoting microtubule stabilization after intracerebral hemorrhage. J Am Heart Assoc 7:e007626.

Zhang B, Carroll J, Trojanowski JQ, Yao Y, Iba M, Potuzak JS, Hogan AML, Xie SL, Ballatore C, Smith AB, Lee VMY, Brunden KR (2012) The microtubule-stabilizing agent, epothilone D, reduces axonal dysfunction, neurotoxicity, cognitive deficits, and Alzheimer-like pathology in an interventional study with aged tau transgenic mice. J Neurosci 32:3601-3611.

Zhao W, Chai Y, Hou Y, Wang DW, Xing JQ, Yang C, Fang QM (2017) Mechanisms responsible for the inhibitory effects of epothilone B on scar formation after spinal cord injury. Neural Regen Res 12:478-485.

Zhu Z, Chuckowree JA, Musgrove R, Dickson TC, Blizzard CA (2020) The pathological outcomes and efficacy of epothilone treatment following traumatic brain injury is determined by age. Neurobiol Aging 93:85-96.

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