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Neuroprotective Efficacy of Estrogen in Experimental Spinal Cord Injury in Rats

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Abstract

Spinal cord injury (SCI) leads to neurological deficit and motor dysfunction. Methylprednisolone, the only drug used for treating SCI, renders limited neuroprotection and remains controversial. Estrogen is one of the most potent multi-active neuroprotective agents and it is currently under investigation in our laboratory for its efficacy in SCI. The present review briefly summarizes our earlier findings on the therapeutic potential of pharmacological/supraphysiological levels of estrogen in SCI and outlines our ongoing research, highlighting the efficacy of physiological levels of estrogen against neuronal injury, axonal degeneration, and gliosis and also the molecular mechanisms of such neuroprotection in experimental SCI. Furthermore, our ongoing studies designed to explore the different translational potential of estrogen therapy suggest that this multi-active steroid may act as an adjunct therapy to promote angiogenesis, thus enhancing the functional recovery following chronic SCI. Taken together, these studies confirm that estrogen is a potential therapeutic agent for treating SCI.

Keywords

axonal degeneration; calpain; estrogen; neuroprotection; spinal cord injury

Introduction

Spinal cord injury (SCI) is a serious neurotrauma that leads to lifelong disability for which no suitable therapeutic strategies exist so far.[1] In the US there are approximately 450,000 people living with SCI, and an additional 11,000 new SCI cases occur every year; the majority being males between the ages of 16-30, as reported by the Foundation for Spinal Cord Injury Prevention, Care, and Cure. Thus, SCI is sufficiently prevalent, highly debilitating, and life-changing incident in otherwise healthy young individuals. Improvement of function in survivors of SCI is an enormous biomedical challenge not only to the clinicians, but also to the researchers.

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Conflicts of Interest
The authors declare no conflicts of interest.

An incomplete SCI is initially mild and causes minimal damage to the spinal cord (SC). However, this injury leads to complex pathologic processes, resulting in damaging secondary SCI. The physiology of secondary damage following SCI depicts deregulated intracellular and extracellular processes, initiating within minutes and propagating through hours and weeks after the primary assault. The final neurological deficit is determined by the extent of severity in which secondary injury deleteriously affects the healthy tissue and aggravates the primary damage. Hence, the main goal of any pharmacological treatment in acute SCI is to prevent and mitigate the detrimental secondary injury pathways, including the loss of cells due to apoptosis. Apoptosis or reversible death process in cells occurring in the periphery of lesion or penumbra is a tightly regulated physiological phenomenon and hence, treatable, as opposed to necrosis, which is an irreversible cell death process occurring within the lesion. Promotion of repair to the chronically injured SC and recovery of neurological function is the primary goal, but this is largely dependent on how early and effectively the primary care is rendered. Thus, the neuroprotective drugs hold enormous promises in the treatment of SCI.

Significant advances in experimental research have led to tremendous progress in unraveling the nature of SCI in the last few decades. A major focus over the years has been to use the corticosteroid methylprednisolone as a therapeutic agent for the treatment of SCI in humans. Unfortunately, data available from different clinical trials are controversial and qualitative.[2, 3] Methylprednisolone treatment in SCI failed to get approval by the Food and Drug Administration that recommended it only as an “optional” treatment by “Guidelines for the Management of Acute Cervical Spine and Spinal Cord Injuries”.[4] Besides, none of the treatments such as thyroid releasing hormone, opioid antagonists, and the free radical scavengers in preclinical and clinical studies have been proven to be a major advantage in treatment of SCI.[5] The major barrier in development of effective SCI therapy lies in the complexity of multiple interlinked and interdependent damaging pathways involved in propagating the secondary injury process following SCI. Researchers have repeatedly emphasized the desperate need for the development of effective SCI therapeutic measures with either a combination of multiple neuroprotectants or with a single multi-active agent. Growing scientific evidence supports the hypothesis that estrogen is such a multi-active agent and may reduce inflammatory responses and increase viability and functionality of damaged neurons in the brain.[6-8] Recent results from our laboratory have further opened the possibility that estrogen therapy can be an efficient means to treat SCI in rats.[9, 10]

Estrogen as a Multi-active Neuroprotectant

Multimodal neuroprotective efficacy of estrogen stems from diverse, yet interlinked mechanisms by which estrogenic steroids produce biological effects.[11] Classically, estrogen signals through a nuclear receptor, which targets transcription of mRNA and cognate protein expression.[12] Estrogen can also act via activation of its receptors ER-α and ER-ß and downstream intracellular signaling through kinases.[6] It can act as an anti-inflammatory agent[13] and promote growth of micro vessels.[14] Estrogen can act directly at neurotransmitter receptor complexes or at ion channels resulting in altered neuronal current conductance or trans-cellular ion flux.[15] Thus, the hormone initiates generalized signaling pathways to the nuclear or membrane-localized effectors. Crosstalk between nuclear activation and membrane-associated events is likely. Estrogen also has important non-cell type-specific actions, such as anti-oxidation and conservation of endogenous free radical scavenging agents.[16] In a nutshell, diverse mechanisms of estrogen mediated neuroprotection may include genomic, receptor-dependent transcriptional regulation, and non-genomic rapid effects that may or may not be receptor mediated but involves regulation by kinases as well as other effects such as anti-inflammatory, anti-oxidative, and anti-

apoptotic, making it a versatile neuroprotectant. Thus, estrogen may be a potential candidate and testing its efficacy against neurotrauma including SCI may be beneficial.

Furthermore, it is important to note that both ER-α and ER-β are distributed widely in the body in both genders. ER-α predominates in the uterus and mammary gland, whereas ER-β has significant roles in the central nervous, cardiovascular, and immune systems.[17] Such wide-scale distribution of the estrogen receptors infers greater access of low dose of estrogen to the receptors and underscores the possibility of beneficial effects of estrogen.

Estrogen Protects Different Neural Cells

Since an array of neural cells is damaged during the progression of SCI lesion, we tested the mechanisms of estrogen efficacy individually in monocultures and subsequently in neuron and glia co-cultures (our unpublished observations). Previous reports from our laboratory have shown that supraphysiological doses of estrogen could combat multiple aspects of oxidative stress through a receptor independent mechanism in a rat astroglial cell line (C6) that resembles primary astrocytes.[18] Furthermore, C6 cells were shown to express estrogen receptors and were protected from glutamate-induced excitotoxic assault by treatment with nanomolar (nM) concentration of estrogen via receptor-mediated mechanism.[19] Likewise, low dose estrogen treatment also ameliorated apoptotic shrinkage and rendered functional protection to primary neuronal cultures exposed to glutamate toxicity, as confirmed by electrophysiological recordings of capacitance.[20] When co-cultured with glia, neurons could tolerate greater glutamate toxicity and estrogen treatment enhanced the protection through receptor-mediated mechanism (our unpublished observations).

Estrogen Efficacy in Experimental SCI

Extending our cell culture findings to experimental SCI in rats, we observed protection of the injured cord in acute phase.[9, 10] Supraphysiological levels of estrogen attenuated inflammation, reduced or restricted the lesion volume, prevented axonal degeneration, and preserved myelin in acute experimental SCI. Moreover, the profound proteolytic events of the Ca^{2+}-activated proteinase calpain were reduced which prevented the apoptosis of neurons largely present in caudal penumbra by estrogen treatment when administered immediately after SCI in rats.[9, 10] In addition, similar high dose estrogen could mitigate the damage and restore functionality in chronic SCI in rats.[21] Such neuroprotective studies paved the path for further investigation of the clinical relevance on the efficacy of lower physiological doses of estrogen, applied immediately and at different times in the acute SCI paradigm. Subsequently, results from these studies helped to explore low dose estrogen efficacy in chronic SCI in rats. Indeed, estrogen mediated neuroprotection in SCI in rats was attained at much lower doses in subsequent studies in our laboratory.

Other protective effects of estrogen administration following SCI thus far include prevention of astrogliosis and microgliosis, reduction of proteolytic and apoptotic markers, and preservation of the axon-myelin structural unit. The estrogen-mediated attenuation of all these parameters is essential and important for recovery of neurological function following SCI. Functional recovery may be enhanced further by promoting microvessel growth and restoring blood supply, needed for cell survival as cells may die due to ischemia caused by disruption of blood vessels following the primary injury to SC. Since estrogen is known to promote angiogenesis and microvessel growth, its administration may help protect cells from ischemic damage following SCI. One of our goals is also to explore the angiogenic mechanism in both acute and chronic SCI.

Apart from our findings there are a few studies from other laboratories that have addressed the estrogen efficacy in SCI. An increase in expression of the estrogen receptors ER-α and

ER-β mRNA in lumbar SC motoneurons has been reported after axomotomy following sciatic nerve crush injury in bilaterally ovariectomized mice where exogenously supplied estrogen capsules (24 μg) rendered a sustained supraphysiological level of serum estrogen for the first three weeks.[22] Estrogen treatment induced gene expression resulting in acceleration of the growth and maturation of the axons. Furthermore, estrogen receptors were transported from the perikaryon into regenerating neurites, where they promoted local regeneration through the non-genomic ERK-activated signaling pathway.[22] Such protective effects of estrogen on motoneurons reflected well in experimental SCI in which it improved functional recovery in the injured rat, in part, by reducing apoptotic cell death with estrogen pretreatment (3-300 μg).[23] Furthermore, in a post-treatment paradigm in the same study, male rats were given a single injection of estrogen (100 μg/kg) immediately post-injury, which also showed significant recovery in locomotor activity coupled with decreased morphologic outcome. Subsequently adopting a 1 h post-SCI treatment regimen, the same group of researchers confirmed the steroid's neuroprotective mechanism being partly mediated by induction of Bcl-2 through PI3K/Akt-dependent CREB activation.[24] Estrogen also reduced the severity of autonomic dysfunction in SCI in male mice with administration of physiological dosage of estrogen in mice, wherein involvement of non-central/non-spinal mechanisms has been suggested.[25]

Protection by estrogen was further confirmed in SCI induced by complete crush injury. Estrogen effects in such severe SCI were assessed by comparing non-ovariectomized, ovariectomized control, and ovariectomized with low physiological level estrogen supplementation (corresponding to 20 pg/ml in blood) in premenopausal and postmenopausal female rats. The study reported improved BBB scores, white matter sparing, and lower motor neuron survival by 21-day post injury.[26] Another group reported that pretreatment with estrogen reduced the development of inflammation, tissue injury, neutrophil infiltration, expression of iNOS, COX-2 activity and several apoptotic markers associated with SC trauma[27] whereas we observed similar effects in a more preclinical post-treatment approach.[9, 10], [21]

The estrogen receptor antagonist ICI 182,780 has been used to confirm the estrogen-receptor involvement in neuroprotective action of estrogen following SCI.[27] A separate study by another group, who adopted our previously reported estrogen dosing regimen (a higher 4 mg/kg and a lower 100 μg/kg), highlighted a transient neuroprotective window through which estrogen protected SC by stimulating early cytokines release and astroglial responses.[28] These investigators suggested that such stimulations might prevent the spread of lesion and retard inflammatory cells to migrate into the surrounding tissue during the critical first week following SCI. The study reported improved locomotor-recovery over 4 weeks after injury and inferred them as probably the consequence of the transient hike in astroglial reactivity due to estrogen.[28]

As opposed to all the affirmative reports on estrogen efficacy following SCI, a solitary report suggests that gender differences in SCI are not estrogen-dependent and hence estrogen may not provide a viable therapy following SCI.[29] On the contrary, estrogen-related gender differences on the survival of rats following traumatic brain injury has been reported.[30] However, multiple reports on estrogen efficacy in experimental SCI conducted in diverse animal models spanning from acute through chronic models with various dosage regimens and confirming different aspects of neuroprotection certainly are in favor of the multi-active estrogen as a therapeutic agent. The results further validate its use as a promising candidate for the treatment of SCI.

A major challenge is establishment of the minimal effective dose taking into account the gender bias of the SCI victims and discerning the post-injury temporal window in which it

will provide beneficial effect. To this end, our studies on post-injury treatment with estrogen, progressively lowering the dose from supraphysiological to physiological and with post-injury time-point of application, add greatly to the clinical relevance of estrogen as a promising agent in the treatment of SCI.

Conclusion

Although our ultimate goal is to unravel the molecular mechanisms by which estrogen prevents, preserves, and restores the injured SC and to make a coherent platform for preclinical testing, our findings thus far strongly suggests that estrogen can be an appropriate candidate for therapy following SCI. One of the major advantages of estrogen over methylprednisolone is that it is multi-active, angiogenic, and not controversial, and it is a natural endogenous hormone - which makes estrogen an ideal agent for the treatment of SCI.

References

1. Mayo Foundation for Medical Education and Research (MFMER). Spinal Cord Injury. Vol. 2009. Spinal Cord Injury: Causes.
2. Miller SM. Methylprednisolone in acute spinal cord injury: a tarnished standard. Journal of Neurosurgical Anesthesiology. 2008; 20:140–142. [PubMed: 18362778]
3. Rozet I. Methylprednisolone in acute spinal cord injury: is there any other ethical choice? Journal of Neurosurgical Anesthesiology. 2008; 20:137–139. [PubMed: 18362777]
4. Hadley MN, et al. Guidelines for the management of acute cervical spine and spinal cord injuries. Clinical Neurosurgery. 2002; 49:407–498. [PubMed: 12506565]
5. Faden AI, Stoica B. Neuroprotection: challenges and opportunities. Archives of Neurology. 2007; 64:794–800. [PubMed: 17562927]
6. Brann DW, et al. Neurotrophic and neuroprotective actions of estrogen: basic mechanisms and clinical implications. Steroids. 2007; 72:381–405. [PubMed: 17379265]
7. Pozzi S, et al. Estrogen action in neuroprotection and brain inflammation. Annals of the New York Academy of Sciences. 2006; 1089:302–323. [PubMed: 17261778]
8. Simpkins JW, Dykens JA. Mitochondrial mechanisms of estrogen neuroprotection. Brain Research Reviews. 2008; 57:421–430. [PubMed: 17512984]
9. Sribnick EA, et al. Estrogen treatment of spinal cord injury attenuates calpain activation and apoptosis. Journal of Neuroscience Research. 2006; 84:1064–1075. [PubMed: 16902996]
10. Sribnick EA, et al. Estrogen attenuated markers of inflammation and decreased lesion volume in acute spinal cord injury in rats. Journal of Neuroscience Research. 2005; 82:283–293. [PubMed: 16130149]
11. Vasudevan N, Pfaff DW. Non-genomic actions of estrogens and their interaction with genomic actions in the brain. Frontiers in Neuroendocrinology. 2008; 29:238–257. [PubMed: 18083219]
12. Marino M, Galluzzo P, Ascenzi P. Estrogen signaling multiple pathways to impact gene transcription. Current Genomics. 2006; 7:497–508. [PubMed: 18369406]
13. Straub RH. The complex role of estrogens in inflammation. Endocrine Reviews. 2007; 28:521–574. [PubMed: 17640948]
14. Miller VM, Duckles SP. Vascular actions of estrogens: functional implications. Pharmacological Reviews. 2008; 60:210–241. [PubMed: 18579753]
15. Kelly MJ, Levin ER. Rapid actions of plasma membrane estrogen receptors. Trends in Endocrinology and Metabolism. 2001; 12:152–156. [PubMed: 11295570]
16. Nilsen J. Estradiol and neurodegenerative oxidative stress. Frontiers in neuroendocrinology. 2008; 29:463–475. [PubMed: 18275991]
17. Gustafsson JA. Novel aspects of estrogen action. Journal of the Society for Gynecologic Investigation. 2000; 7:S8–S9. [PubMed: 10732321]
18. Sur P, et al. Estrogen attenuates oxidative stress-induced apoptosis in C6 glial cells. Brain Research. 2003; 971:178–188. [PubMed: 12706234]

19. Sribnick EA, Ray SK, Banik NL. Estrogen prevents glutamate-induced apoptosis in C6 glioma cells by a receptor-mediated mechanism. Neuroscience. 2006; 137:197–209. [PubMed: 16289585]
20. Sribnick EA, et al. 17β-Estradiol attenuates glutamate-induced apoptosis and preserves electrophysiologic function in primary cortical neurons. Journal of Neuroscience Research. 2004; 76:688–696. [PubMed: 15139027]
21. Sribnick EA, et al. Estrogen improves locomotor function following spinal cord injury. Journal of Neurochemistry. 2007; 102(Suppl 1):203.
22. Islamov RR, et al. Effect of 17β-estradiol on gene expression in lumbar spinal cord following sciatic nerve crush injury in ovariectomized mice. Brain Research. 2003; 966:65–75. [PubMed: 12646309]
23. Yune TY, et al. Systemic administration of 17β-estradiol reduces apoptotic cell death and improves functional recovery following traumatic spinal cord injury in rats. Journal of Neurotrauma. 2004; 21:293–306. [PubMed: 15115604]
24. Yune TY, et al. Estrogen-induced Bcl-2 expression after spinal cord injury is mediated through phosphoinositide-3-kinase/Akt-dependent CREB activation. Journal of Neurotrauma. 2008; 25:1121–1131. [PubMed: 18785877]
25. Webb AA, et al. Estrogen reduces the severity of autonomic dysfunction in spinal cord-injured male mice. Behavioural Brain Research. 2006; 171:338–349. [PubMed: 16712973]
26. Chaovipoch P, et al. 17β-Estradiol is protective in spinal cord injury in post- and pre-menopausal rats. Journal of Neurotrauma. 2006; 23:830–852. [PubMed: 16774470]
27. Cuzzocrea S, et al. Effect of 17β-estradiol on signal transduction pathways and secondary damage in experimental spinal cord trauma. Shock. 2008; 29:362–371. [PubMed: 17704735]
28. Ritz MF, Hausmann ON. Effect of 17β-estradiol on functional outcome, release of cytokines, astrocyte reactivity and inflammatory spreading after spinal cord injury in male rats. Brain Research. 2008; 1203:177–188. [PubMed: 18316064]
29. Swartz KR, et al. Gender differences in spinal cord injury are not estrogen-dependent. Journal of Neurotrauma. 2007; 24:473–480. [PubMed: 17402853]
30. Roof RL, Hall ED. Estrogen-related gender difference in survival rate and cortical blood flow after impact-acceleration head injury in rats. Journal of Neurotrauma. 2000; 17:1155–1169. [PubMed: 11186229]