The Limited Effects of Estradiol Administration Immediately after Spinal Cord Injury

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Objective: We aimed to investigate estradiol (E2) as a therapeutic drug for spinal cord injury (SCI) and elucidate the disagreement in the field about the use of this hormone after an injury.

Methods: Eleven animals underwent surgery (laminectomy at the T9–T10 levels) followed by an intravenous injection (100 µg) of an E2 bolus and the implantation of 0.5cm of Silastic tubing containing 3 mg of E2 (sham E2 + E2 bolus) immediately after the laminectomy. The SCI control animals received a moderate contusion using the Multicenter Animal SCI Study impactor device over the exposed spinal cord followed by an intravenous bolus injection of sesame oil and were implanted with empty Silastic tubing (injury SE + vehicle); treated rats received a bolus of E2 and a Silastic implant with 3 mg of E2 (injury E2 + E2 bolus). Functional locomotor recovery and fine motor coordination were assessed by the Basso, Beattie, and Bresnahan (BBB) open field test and grid-walking tests, respectively, from the acute (7 days post-injury [DPI]) to the chronic stages (35 DPI). Anatomical studies of the cord were performed using Luxol fast blue staining followed by densitometric analysis.

Results: As observed in the BBB open field and the grid-walking tests, E2 post-SCI did not improve locomotor function but instead increased spared white matter tissue, in the rostral region.

Conclusion: Estradiol post-SCI, at the dose and route of administration used in this study, failed to promote locomotor recovery but partially restored spared white matter tissue. [*P R Health Sci J 2023;42(1):23-28*]

Key words: Trauma, Behavioral locomotor recovery, Spared white matter tissue, Grid walking, Neuroprotection

Spinal cord injury (SCI) is a detrimental condition with a limited prognosis of improvement in locomotor function. Molecular, cellular, and anatomical changes after the trauma influence patient outcome. The loss of white matter tissue after SCI impairs the action potential conduction through the axon, the latter is needed to elicit voluntary movement. Many studies have failed to promote not only the complete regeneration or full myelination of the affected axons but also the reestablishment of spinal cord function after the insult. Importantly, some findings have shown that the sex steroid hormone 17β-estradiol (E2) can serve as a neuroprotective factor in animal models with traumatic injury and provide some locomotor recovery. Several studies have shown that E2 is known to have neuroprotective and remyelination mechanisms. However, the use of E2 as a translational intervention for SCI remains in debate since the preclinical data are contradictory (1–17).

Several publications declined to support the notion that E2 can have neuroprotective effect after SCI. For example, female rats treated with supraphysiological levels of E2 exhibited functional outcomes that were similar to those of control rats after SCI (17). Moreover, the sustained release of E2 did not improve behavioral outcomes after SCI (16) or in ischemia-reperfusion models (15). Finally, variations in physiological levels of E2 by estrous cycle did not improve locomotor recovery after SCI (14). In contrast, E2 treatment prior to SCI promoted functional locomotor recovery and the increased preservation of white matter (13,18–20). A potential explanation for these effects is that E2 activates genomic, non-genomic, and/or antioxidant pathways that confer neuroprotection prior to SCI. Cells in the central nervous system (CNS)—neurons, astrocytes, oligodendrocytes, and

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microglia—as well as immune cells that infiltrate the lesion site express estrogen receptors (ERs) (12,21–23). The possible mechanisms used by E2 to promote a cellular response might be through the ERs (α and ß) that are located in the cytosol or nucleus for genomic activity. Also, E2 may interact with G protein–coupled receptor 30 in the plasma membrane for the activation of non-genomic pathways through G proteins and/or the regulation of protein kinases. The activation of ER-α or ER-ß by E2 could activate as well anti-apoptotic, anti-gliotic, or anti-inflammatory genes through estrogen response elements (5,24–26). A reduction in the activity of the protease caspase-3, which is related to apoptosis, and an increase in the expression of the anti-apoptotic gene B-cell lymphoma 2 were observed when E2 was administered prior to SCI (20). Further, E2 pre-treatment decreased pro-inflammatory cytokine mRNA (tumor necrosis growth factor-α and interleukin 1-ß) expression in the injured spinal cord (19), decreased reactive gliosis (5,26), and reduced autophagic activity (27) and, probably, Akt signaling (28–29). Therefore, E2 may, at the injury site, act on cells from the CNS to promote cell survival, reduce the gliotic response, promote remyelination, and/or reduce inflammation, supporting the notion that this hormone has a neuroprotective effect.

The administration of E2 as a pretreatment for SCI limits the translational potential of these studies. In this study, we aimed to investigate the use of E2 as a therapeutic drug for SCI and elucidate the disagreement in the field about the use of this hormone after trauma to the spinal cord. Immediately after SCI, we administered a 100-µg intravenous bolus of E2 to rapidly increase circulating levels to a supraphysiological concentration. To maintain the supraphysiological levels of E2 for several weeks after the trauma, we implanted Silastic tubes containing 3 mg of E2 (13). This process was different form the one we described previously, which entailed pretreating the rats with E2 for 7 days prior to their being injured (13).

Materials and Methods

Female adult Sprague Dawley rats (200–220 grams) were acquired from Hilltop Lab Animals, Inc. (Scottsdale, PA, USA), and maintained at a 12:12 h light–dark cycle. Rat chow and water were provided ad libitum (Harlan Teklad, Indianapolis, IN, USA). The animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Puerto Rico, Medical Sciences Campus, to minimize the number of animals.

Eleven rats underwent surgery, as previously described (30). The phase of the estrous cycle was not determined since previous studies by Baker and Hagg, 2005, demonstrated that inducing SCI at specific stages of the cycle did not affect the outcome. In addition, the protocol was designed to simulate a real situation, in which the phase of the estrous cycle is unknown at the time of the injury. Sham animals received a laminectomy (T9–T10 level) followed by the intravenous injection of the previously mentioned 100 µg of E2 (in bolus form) and the implantation of a Silastic tube loaded with 3 mg of E2 (sham E2 + E2 bolus), as described by Mosquera et al., 2014. The silastic tubing was placed in the mid-scapular region immediately after laminectomy or SCI. Injured animals received a moderate contusion using the Multicenter Animal SCI Study (MASCIS) impactor device. A 10-gram rod was dropped from a 12.5 mm height over the exposed spinal cord and the compression maintained for 5 seconds. The SCI control animals received an intravenous bolus injection of sesame oil and an implant consisting of a Silastic tube, though empty, in this case (injury SE + vehicle), while the treated rats received a bolus of E2 and a Silastic implant containing 3 mg of E2 (injury E2 + E2 bolus). Previous studies in the laboratory using radioimmunoassays demonstrated that a Silastic tube with 3 mg of E2 was sufficient to elevate the concentration of this hormone to supraphysiological levels with the increase maintaining that level for at least 28 days (31). The animals were treated with buprenorphine (0.05 mg/kg) twice per day for 3 days and with cefazolin (25 mg/kg) for 7 days after surgery. The bladders of the animals were expressed manually until the micturition reflexes were fully reestablished. Throughout the experiment, cereal and proper enrichment (cardboard tubes) were provided to all the animals (sham and injury) (32).

The recovery of locomotor function was assessed by the Basso, Beattie, and Bresnahan (BBB) open field test from the acute (7 days post-injury [DPI]) to the chronic stages (35 DPI). Two evaluators, blinded to the treatment, scored the recovery of hindlimb movement while the affected rats walked in a plastic pool. Each hindlimb was assessed individually, and the average for each animal was reported. The grid-walking test was used to evaluate the effect of E2 on fine motor coordination (dorsolateral, corticospinal, and rubrospinal tracts) as the affected rats attempted to cross a 3-foot-long horizontal ladder with randomly spaced rungs (30,32,33). The evaluators recorded the number of errors (the rats misplacing their hindlimbs between the rungs), and the average was reported.

At 35 DPI, the rats were euthanized by intracardiac perfusion with cold phosphate buffered saline followed by cold 4% paraformaldehyde. Approximately 3 cm of the spinal cord tissue containing the rostral and epicentral areas was collected, cryoprotected, and sectioned, as described previously (33). Representative sections from the rostral and epicentral regions of the cord were collected and stained with 0.01% Luxol fast blue, followed by densitometric analysis (33). All the results presented are the mean and standard error of the mean. The results were considered significantly different if P was less than .05. Because of the limited number of samples that we had for the behavioral data, non-parametrical Kruskal–Wallis tests were performed. Densitometry analysis and quantification experiments were analyzed by 1-way analysis of variance (ANOVA) followed by the Tukey multiple comparison test.

Results

Behavioral analyses demonstrated that the treatment of injured rats with E2 after SCI did not improve locomotor recovery (Fig. 1A). The sham animals (n = 3) had perfect BBB open field test scores during the study. The injured control rats (n = 3) lost significant locomotor function over time compared to the sham animals. Importantly, E2 treatment (n = 6 for 0 and 7 DPI; n = 5 for 14–35 DPI) immediately after SCI failed to improve locomotor recovery. The Kruskal–Wallis tests revealed no significance between injury groups treated and not treated with E2 [H (2) = 6.586, and P = .6209 for 7 DPI; H (2) = 6.189, and P = .8327 for 14 DPI; H (2) = 6.14, and P > .9999 for 21 DPI; H (2) = 6.338, and P = .6560 for 28 DPI; and H (2) = 6.123, and P = .91115 for 35 DPI]. A grid-walking analysis (Fig. 1B) revealed that E2 treatment was unsuccessful in terms of improving fine motor coordination. No significant difference was observed between the injured animals treated with E2 (n = 6 for 0 and 7 DPI; n = 5 for 14–35 DPI) and those that were injured but remained untreated [n = 3: 0 DPI: H (2) = 2.75, and P = .3384; 7 DPI: H (2) = 8.323, and P = .5081; 14 DPI: H (2) = 6.137, and P = .7406; 21 DPI: H (2) = 6.137, and P = .7406; 28 DPI: H (2) = 6.338, and P = .6560; and 35 DPI: H (2) = 6.104, and P = .8249]. Similar results were obtained with the beam-crossing assay, confirming the absence of behavioral locomotor recovery when E2 was administered after SCI (data not shown).

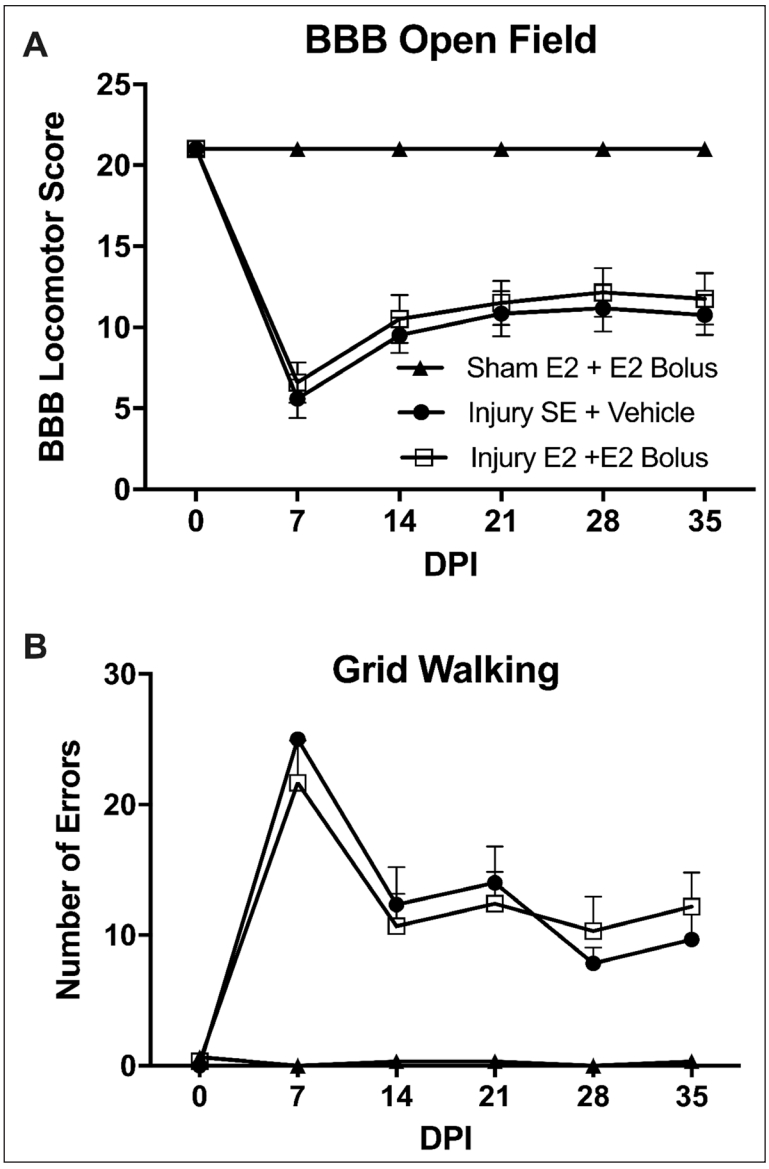

Figure 1. Estradiol (E2) administration after spinal cord injury (SCI) failed to enhance functional locomotor recovery. (A) BBB scores revealed that the administration of 3 mg of E2 combined with an intravenous injection of 100 µg of E2 (injury E2 + E2 bolus) immediately after injury was not capable of enhancing functional locomotor recovery compared to the injury control group (injury SE + vehicle). Kruskal–Wallis revealed no significance between injury groups treated and not treated with E2 at 7, 14, 21, 28, and 35 DPI. (B) A similar pattern was observed on the grid-walking tests, in which no significant difference was observed between injured animals treated with E2 and those that were injured but untreated at all the time points studied. The ns for the groups in both tests, BBB and grid walking, are as follows: sham E2 + E2 bolus = 3; injury SE + vehicle = 3; and for injury E2 + E2 bolus = 6 (0 and 7 DPI) and 5 (14–35 DPI).

Spared white matter tissue was analyzed by densitometry for Luxol fast blue staining (Fig. 2). In the rostral areas, SCI significantly decreased the amount of spared white matter tissue compared to sham (sham 816 ± 27 pixels/area vs injury vehicle 523 ± 65 pixels/area). Estradiol treatment favored the amount of spared white matter tissue in the rostral area (injury E2 771 ± 61 pixels/area vs injury vehicle 523 ± 65 pixels/area) but not the lesion epicenter (injury E2 482 ± 115 pixels/area vs injury vehicle 315 ± 38 pixels/area) or caudal segments (injury E2 702 ± 56 pixels/area vs injury vehicle 689 + 102 pixels/area). One-way ANOVA $F_{(2,6)} = 8.581$,* = P < .05; P = .0174; n = 3 followed by a Tukey's post hoc test (P < .04); for epicenter segments: 1-way ANOVA $F_{(2,6)} = 12.92$, P = .0067; and for caudal segments: 1-way ANOVA $F_{(2,6)} = 2.416$, P = .1700 (n = 3, for all groups) demonstrated a significant difference in the rostral area. Further analysis showed that in the epicentral area, the injured rats displayed a significant decrease in the amount of white matter (315 ± 38 pixels/area) relative to the sham animals (836 ± 42 pixels/area). The lack of neuroprotective effect was also evident in the caudal areas of the injured animals treated with E2 (702 ± 56 pixels/area) compared to the injured animals in the vehicle group (689 ± 102 pixels/area); and, finally, the animals in the sham group retained their white matter in this region (893 ± 52 pixels/area).

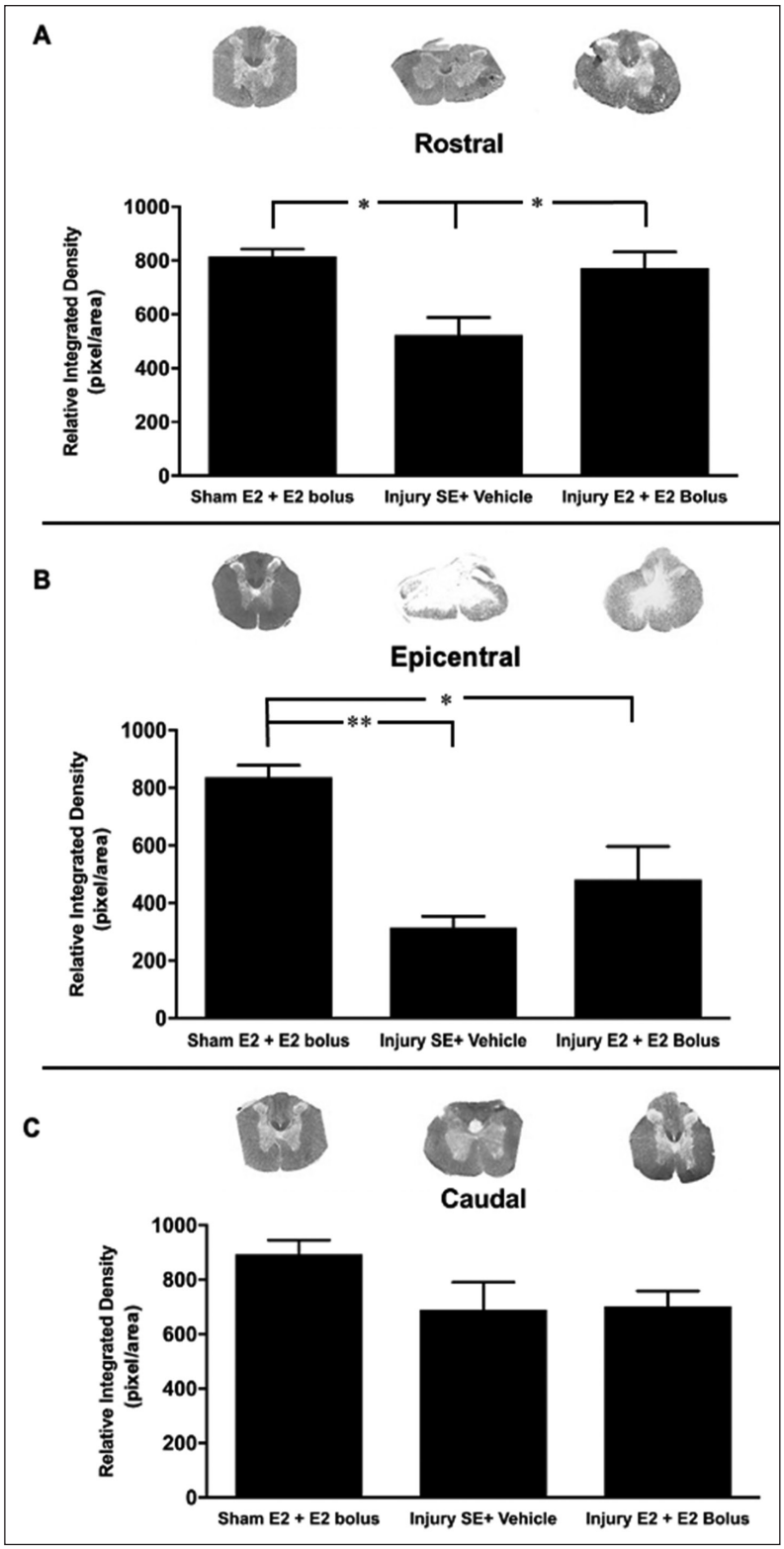

Figure 2. Estradiol increased white matter preservation after SCI. Estradiol administration immediately after SCI increased spared white matter tissue rostral to the lesion's epicenter compared to what was seen in the control group (A). One-way ANOVA followed by Tukey's post hoc test confirmed the significant difference among these groups (n = 3). The effect of E2 was not observed in the epicentral (B) or caudal (C) segments of the cord after the injury (n = 3).

Discussion

A wide range of changes at the molecular and cellular level impede axonal extension or cell survival after SCI (32). Our group and others have shown that E2 pretreatment is sufficient to result in the promotion of locomotor function and white matter preservation after SCI occurs (13). In this study, we investigated the effect of E2 on the injured cord when administered immediately after the contusion occurred. The behavioral tests in this study indicate that the immediate administration of supraphysiological levels of this neuroprotective hormone by an intravenous bolus followed by its continuous release via Silastic implants failed to promote locomotor recovery. It is possible that the molecular and cellular events after SCI occur quickly, diminishing the time available for E2 to reach deep areas of the spinal cord and initiate a neuroprotective response. Estradiol may act via a genomic route to activate estrogen response genes, which are deemed neuroprotective, but these mechanisms rely on time to assemble the genomic machinery (19,20).

Interestingly, anatomical studies confirmed that E2 treatment after SCI significantly preserves white matter in segments rostral to the lesion epicenter. Our group observed similar regional effects of E2 in decreasing the presence of radical oxygen species only in the rostral regions when E2 was administered prior to SCI (13). In the pretreatment studies published by our group and others, the spinal cord (and other tissues) was already in the presence of this neuroprotective hormone at the time of the injury. In this study, the administered (after SCI) E2 took time to reach the damaged cells and initiate a robust neuroprotective response that was similar to what was observed before. One point to be considered for an increase in spared white matter tissue rostral to the lesion epicenter without a behavioral recovery is the effect of E2 on myelin formation. This hormone may help oligodendrocytes to survive after SCI and maintain some Luxol staining levels similar to the treated (control) rats. However, appropriate myelination is critical to maintaining axon function. Therefore, future experiments should analyze the effect of E2 on proper myelin organization around the axons. Finally, differences between the absence of behavioral locomotor results obtained in this project and those observed by

others may be due to the injury models used (NYU/MASCIS impactor, Infinite Horizon impactor, crush injury with vascular or aneurysm clips, micro-dissector forceps, hemisection or transection), SCI level (C5, T2, T6–7, T8, T8–T9, T10, or T12), degree of compression (12.5–80 mm impactor height if using a weight-drop impactor or 100–190 kdyn if using the Infinite Horizon impactor), dose of E2 (0.001–4 mg/kg E2 encapsulated in cyclodextrin or dissolved in dimethyl sulfoxide, μg/ml–1 mg/ml in silastic capsules, or a 0.25–5 mg E2 pellet), rat strain (Sprague Dawley, Wistar, Lewis, or Fischer), rat strain (CD1 or C5BL/6 or B6/129 mice), delivery system (injection, mini-pumps, commercial pellets, or silastic tubing), and sex of the animal used (4,34,35). Nevertheless, using the same animal model (Sprague Dawley rats), gender (female), injury model (NYU impactor device), moderate compression injury (10 grams from a 12.5 mm height), dose of E2 (3 mg), and delivery system (Silastic implants), we obtained a different result than previously published by our group when the rats were pretreated with E2 for a week before the injury (13). The data presented support the limited effect of E2 if administered after SCI (instead of before) and should solve the disagreement within the field about the possible effect and use of E2 after spinal cord trauma.

In conclusion, the results obtained support the notion that E2 has a reduced neuroprotective effect of E2 in the context of CNS trauma. The results obtained confirm the restricted effect of E2 if administered after SCI and help to shed light on the disagreement in the field about the possible beneficial effects of this hormone after SCI. However, the timing, dose, and mechanism of administration that are required for the drug to be effective should be considered, though it should be noted that detrimental or adverse side-effects may arise with higher doses of E2 (36). Therefore, alternative treatments that interact with ERs or major estrogenic pathways may have positive outcomes that should be evaluated for SCI treatment.

Resumen

Objetivo: Investigar el uso de estradiol (E2) como tratamiento para una lesión del cordón espinal (LCE) y elucidar la discrepancia en el campo sobre el uso de esta hormona luego de un daño. Métodos: Once animales fueron sometidos a cirugía, el grupo control (laminectomía a nivel T9-T10), seguido por inyección intravenosa (100 μg) de E2 y 0.5 cm de tubo de Silástico conteniendo 3 mg de E2 (Control E2 + Bolo E2) inmediatamente después de la laminectomía. Animales lesionados control, recibieron una contusión moderada utilizando el MASCIS (por sus siglas en inglés) sobre la médula espinal expuesta seguido de una inyección intravenosa de aceite de sésamo y un tubo Silástico vacío (Lesión SE + Vehículo), ratas en el grupo experimental recibieron bolo de E2 e implante de Silástico (3 mg de E2; Lesión E2 + Bolo E2). La recuperación funcional locomotora y la coordinación motora fina se evaluaron mediante pruebas de campo abierto BBB y caminado de cuadrícula, respectivamente, desde etapas agudas (7 days post-injury [DPI]) hasta etapas crónicas (35 DPI). Los estudios anatómicos del cordón se realizaron utilizando la tinción Luxol Fast Blue seguido de análisis densitométrícos. Resultados: En las pruebas de BBB en un espacio abierto y de caminado de cuadrícula, el E2 post-LCE no promovió recuperación locomotora, pero aumentó el tejido preservado de materia blanca en la región rostral. Conclusión: E2 post-LCE, en la dosis y vía de administración utilizadas en este estudio, no promovieron recuperación locomotora, pero restauraron parcialmente el tejido preservado de la materia blanca.

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References

1. Kachadroka S, Hall AM, Niedzielko TL, Chongthammakun S, Floyd CL. Effect of endogenous androgens on 17beta-estradiol-mediated protection after spinal cord injury in male rats. J Neurotrauma. 2010;27(3):611-626. doi:10.1089/neu.2009.1069
2. Ritz MF, Hausmann ON. Effect of 17beta-estradiol on functional outcome, release of cytokines, astrocyte reactivity and inflammatory spreading after spinal cord injury in male rats. Brain Res. 2008;1203:177-188. doi:10.1016/j.brainres.2008.01.091
3. Samantaray S, Sribnick EA, Das A, et al. Neuroprotective efficacy of estrogen in experimental spinal cord injury in rats. Ann N Y Acad Sci. 2010;1199:90-94. doi:10.1111/j.1749-6632.2009.05357.x
4. Samantaray S, Smith JA, Das A, et al. Low dose estrogen prevents neuronal degeneration and microglial reactivity in an acute model of spinal cord injury: effect of dosing, route of administration, and therapy delay. Neurochem Res. 2011;36(10):1809-1816. doi:10.1007/s11064-011-0498-y
5. Siriphorn A, Dunham KA, Chompoopong S, Floyd CL. Postinjury administration of 17β-estradiol induces protection in the gray and white matter with associated functional recovery after cervical spinal cord injury in male rats. J Comp Neurol. 2012;520(12):2630-2646. doi:10.1002/cne.23056
6. Sribnick EA, Wingrave JM, Matzelle DD, Ray SK, Banik NL. Estrogen as a neuroprotective agent in the treatment of spinal cord injury. Ann N Y Acad Sci. 2003;993:125-160. doi:10.1111/j.1749-6632.2003.tb07521.x
7. Sribnick EA, Wingrave JM, Matzelle DD, Wilford GG, Ray SK, Banik NL. Estrogen attenuated markers of inflammation and decreased lesion volume in acute spinal cord injury in rats. J Neurosci Res. 2005;82(2):283-293. doi:10.1002/jnr.20622
8. Sribnick EA, Ray SK, Banik NL. Estrogen prevents glutamate-induced apoptosis in C6 glioma cells by a receptor-mediated mechanism. Neuroscience. 2006;137(1):197-209. doi:10.1016/j.neuroscience.2005.08.074
9. Sribnick EA, Samantaray S, Das A, et al. Postinjury estrogen treatment of chronic spinal cord injury improves locomotor function in rats. J Neurosci Res. 2010;88(8):1738-1750. doi:10.1002/jnr.22337
10. Sribnick EA, Del Re AM, Ray SK, Woodward JJ, Banik NL. Estrogen attenuates glutamate-induced cell death by inhibiting Ca2+ influx through L-type voltage-gated Ca2+ channels. Brain Res. 2009;1276:159-170. doi:10.1016/j.brainres.2009.04.022
11. Tan H, Tang Y, Li J, He T, Zhou M, Hu S. Prognosis Evaluation Using 18F-Alfatide II PET in a Rat Model of Spinal Cord Injury Treated With Estrogen. Mol Imaging. 2020;19:1536012120909199. doi:10.1177/1536012120909199

12. Hu R, Sun H, Zhang Q, et al. G-protein coupled estrogen receptor 1 mediated estrogenic neuroprotection against spinal cord injury. Crit Care Med. 2012;40(12):3230-3237. doi:10.1097/CCM.0b013e3182657560
13. Mosquera L, Colón JM, Santiago JM, et al. Tamoxifen and estradiol improved locomotor function and increased spared tissue in rats after spinal cord injury: their antioxidant effect and role of estrogen receptor alpha. Brain Res. 2014;1561:11-22. doi:10.1016/j.brainres.2014.03.002
14. Baker KA, Hagg T. An adult rat spinal cord contusion model of sensory axon degeneration: the estrus cycle or a preconditioning lesion do not affect outcome. J Neurotrauma. 2005;22(4):415-428. doi:10.1089/neu.2005.22.415
15. Cavalcante LP, Ferreira SG, Pereira DR, et al. Acute administration of oestradiol or progesterone in a spinal cord ischaemia-reperfusion model in rats. Interact Cardiovasc Thorac Surg. 2018;26(2):196-201. doi:10.1093/icvts/ivx314
16. Sengelaub DR, Han Q, Liu NK, et al. Protective Effects of Estradiol and Dihydrotestosterone following Spinal Cord Injury. J Neurotrauma. 2018;35(6):825-841. doi:10.1089/neu.2017.5329
17. Swartz KR, Fee DB, Joy KM, et al. Gender differences in spinal cord injury are not estrogen-dependent. J Neurotrauma. 2007;24(3):473-480. doi:10.1089/neu.2006.0167
18. Chaovipoch P, Jelks KA, Gerhold LM, West EJ, Chongthammakun S, Floyd CL. 17beta-estradiol is protective in spinal cord injury in post- and pre-menopausal rats. J Neurotrauma. 2006;23(6):830-852. doi:10.1089/neu.2006.23.830
19. Cuzzocrea S, Genovese T, Mazzon E, et al. Effect of 17beta-estradiol on signal transduction pathways and secondary damage in experimental spinal cord trauma. Shock. 2008;29(3):362-371. doi:10.1097/shk.0b013e31814545dc
20. Yune TY, Kim SJ, Lee SM, et al. Systemic administration of 17beta-estradiol reduces apoptotic cell death and improves functional recovery following traumatic spinal cord injury in rats. J Neurotrauma. 2004;21(3):293-306. doi:10.1089/089771504322972086
21. Arvanitis DN, Wang H, Bagshaw RD, Callahan JW, Boggs JM. Membrane-associated estrogen receptor and caveolin-1 are present in central nervous system myelin and oligodendrocyte plasma membranes. J Neurosci Res. 2004;75(5):603-613. doi:10.1002/jnr.20017
22. Giraud SN, Caron CM, Pham-Dinh D, Kitabgi P, Nicot AB. Estradiol inhibits ongoing autoimmune neuroinflammation and NFkappaB-dependent CCL2 expression in reactive astrocytes. Proc Natl Acad Sci U S A. 2010;107(18):8416-8421. doi:10.1073/pnas.0910627107
23. Wu WF, Tan XJ, Dai YB, Krishnan V, Warner M, Gustafsson JÅ. Targeting estrogen receptor β in microglia and T cells to treat experimental autoimmune encephalomyelitis. Proc Natl Acad Sci U S A. 2013;110(9):3543-3548. doi:10.1073/pnas.1300313110
24. Brunsing RL, Owens KS, Prossnitz ER. The G protein-coupled estrogen receptor (GPER) agonist G-1 expands the regulatory T-cell population under TH17-polarizing conditions. J Immunother. 2013;36(3):190-196. doi:10.1097/CJI.0b013e31828d8e3b
25. Rettew JA, McCall SH 4th, Marriott I. GPR30/GPER-1 mediates rapid decreases in TLR4 expression on murine macrophages. Mol Cell Endocrinol. 2010;328(1-2):87-92. doi:10.1016/j.mce.2010.07.017
26. Rozovsky I, Wei M, Stone DJ, et al. Estradiol (E2) enhances neurite outgrowth by repressing glial fibrillary acidic protein expression and reorganizing laminin. Endocrinology. 2002;143(2):636-646. doi:10.1210/endo.143.2.8615
27. Lin CW, Chen B, Huang KL, Dai YS, Teng HL. Inhibition of Autophagy by Estradiol Promotes Locomotor Recovery after Spinal Cord Injury in Rats. Neurosci Bull. 2016;32(2):137-144. doi:10.1007/s12264-016-0017-x
28. Jover-Mengual T, Miyawaki T, Latuszek A, Alborch E, Zukin RS, Etgen AM. Acute estradiol protects CA1 neurons from ischemia-induced apoptotic cell death via the PI3K/Akt pathway. Brain Res. 2010;1321:1-12. doi:10.1016/j.brainres.2010.01.046
29. Yune TY, Park HG, Lee JY, Oh TH. Estrogen-induced Bcl-2 expression after spinal cord injury is mediated through phosphoinositide-3-kinase/Akt-dependent CREB activation. J Neurotrauma. 2008;25(9):1121-1131. doi:10.1089/neu.2008.0544
30. Santiago JM, Rosas O, Torrado AI, González MM, Kalyan-Masih PO, Miranda JD. Molecular, anatomical, physiological, and behavioral studies of rats treated with buprenorphine after spinal cord injury. J Neurotrauma. 2009;26(10):1783-1793. doi:10.1089/neu.2007.0502
31. Mosquera L, Shepherd L, Torrado AI, Torres-Diaz YM, Miranda JD, Segarra AC. Comparison of Two Methods of Estradiol Replacement: their Physiological and Behavioral Outcomes. J Vet Sci Technol. 2015;6(6):276. doi:10.4172/2157-7579.1000276
32. Colón JM, Torrado AI, Cajigas Á, et al. Tamoxifen Administration Immediately or 24 Hours after Spinal Cord Injury Improves Locomotor Recovery and Reduces Secondary Damage in Female Rats. J Neurotrauma. 2016;33(18):1696-1708. doi:10.1089/neu.2015.4111
33. Colón JM, González PA, Cajigas Á, et al. Continuous tamoxifen delivery improves locomotor recovery 6h after spinal cord injury by neuronal and glial mechanisms in male rats. Exp Neurol. 2018;299(Pt A):109-121. doi:10.1016/j.expneurol.2017.10.006
34. Letaif OB, Cristante AF, Barros Filho TE, et al. Effects of estrogen on functional and neurological recovery after spinal cord injury: An experimental study with rats. Clinics (Sao Paulo). 2015;70(10):700-705. doi:10.6061/clinics/2015(10)08
35. Samantaray S, Das A, Matzelle DC, et al. Administration of low dose estrogen attenuates persistent inflammation, promotes angiogenesis, and improves locomotor function following chronic spinal cord injury in rats. J Neurochem. 2016;137(4):604-617. doi:10.1111/jnc.13610
36. Gottipati MK, Ellman SAT, Puhl DL, et al. Acute Dose-Dependent Neuroprotective Effects of Poly(pro-17β-estradiol) in a Mouse Model of Spinal Contusion Injury. ACS Chem Neurosci. 2021;12(6):959-965. doi:10.1021/acschemneuro.0c00798