PERSPECTIVE

Future directions for using estrogen receptor agonists in the treatment of acute and chronic spinal cord injury

All synthetic and natural estrogen receptor agonists, including the most potent physiological molecule estrogen or estradiol (E2), work typically *via* activation of nuclear estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ). Both ERα and ERβ modulate the expression of a variety of genes in the cells. Neurons and glial cells express ERα and ERβ. Many studies so far from our and other laboratories have firmly established the mode of actions that ERα and ERβ agonists are very promising anti-inflammatory and neuroprotective agents in the treatment of neurodegenerative diseases and injuries including spinal cord injury (SCI) (Chakrabarti et al., 2014a). There are selective ERα agonists and also selective ERβ agonists but E2 works *via* both ERα and ERβ to mediate its anti-inflammatory and neuroprotective effects. Our studies show that E2 is a better therapeutic agent than a selective ERα agonist such as 1,3,5-tris (4-hydroxyphenyl)-4-propyl-1H-pyrazole and a selective ERβ agonist such as 2,3-bis (4-hydroxyphenyl) propionitrile or Way 200070 to provide functional neuroprotection in cell culture models of SCI (Das et al, 2011; Chakrabarti et al., 2014b). We have also recently reported that E2 even at a low dose provides highly significant therapeutic efficacy for amelioration of neurodegeneration and recovery of locomotor function in animal models of acute SCI (Samantaray et al., 2016a) and chronic SCI (Samantaray et al., 2016b). Future studies for using E2 in the treatment of SCI in animal models need to be focused on optimal enhancement of its multi-active therapeutic effects at a physiologically relevant further low dose, may be, in combination with another therapeutic agent.

Although there are three main naturally occurring estrogens such as estrone (E1), E2, and estriol (E3), E2 is the most prevalent and highly potent steroidal sex hormone in females. Being the primary sex hormone in females, E2 plays not only the vital roles in remodeling and regulation of the reproductive system but it also contributes to cognitive function in the central nervous system (CNS). When compared with females, E2 level is significantly low in males. However, E2 even at a very low level has important roles in maintaining overall health in males. Because E2 can easily pass through the blood-brain barrier and diffuse across the cell membrane, it is readily available to bind to and activate the nuclear ERα and ERβ to modulate expression of many target genes for anti-inflammatory and neuroprotective effects in the CNS cells following a CNS injury such as SCI. Relatively more E2 level in female mice than male mice can provide an advantage to recovery of locomotor function following SCI (Farooque et al., 2006). The most mesmerizing fact about E2 is that it is a multi-active therapeutic agent in SCI, the pathogenesis of which activates multiple detrimental factors and pathways. It is now clear from our recent results that E2 is a much better therapeutic option in preclinical animal models of both acute SCI (Samantaray et al., 2016a) and chronic SCI (Samantaray et al., 2016b) than the currently recommended pharmacotherapy with the high dose of methylprednisolone, which is used only in acute SCI in humans and has severe side effects. Various studies have already revealed that the multi-active E2 has many well-known mechanisms to prevent progressive pathogenesis and promote recovery of locomotor function in SCI (Ray et al., 2011; Chakrabarti et al., 2016). The mode of actions of E2 includes inhibition of oxidative stress, neuroinflammation, demyelination, intracellular free Ca^{2+}, pro-apoptotic Bax protein, and cysteine proteases (calpain and caspases) of the apoptotic pathways but E2 is also an inducer of anti-apoptotic Bcl-2 protein, growth factors and cell proliferation, and angiogenic factors and angiogenesis for recovery of locomotor function in both acute SCI and chronic SCI (Samantaray et al., 2016a, Samantaray et al., 2016b).

Earlier results from our and other laboratories reported that supra-physiological high dose of E2 worked as a very effective anti-oxidant and showed high efficacy in the treatment of SCI in animals. Therefore, it may be provocative to use supra-physiological high dose of E2 to maximize its anti-oxidant effects in the treatment of SCI in humans as well. But use of high dose of E2 for a long time can be associated with risks of several cancers in females (Benyi et al., 2014) and also promotion of conspicuous feminine characteristics in males. In view of these concerns, a high dose of E2 should not be translated to the clinics for treatment of SCI in humans. Our recent results showed that a low dose (10 or 100 μg/kg) of E2 reduced reactive gliosis, Bax/Bcl-2 ratio, expression and activity of calpain and caspase-3, neuronal death in lesion and caudal regions of the spinal cord in acute SCI rats (Samantaray et al., 2016a). Treatment of animals with either of these low doses of E2 reduced pro-inflammatory factors and cysteine protease activities to protect neurons in acute SCI. Interestingly, our data clearly demonstrated that E2 at 10 μg/kg was as effective as at 100 μg/kg in improving these parameters in acute SCI animals. Thus, we suggested that E2 at a low dose (10 μg/kg) could be a promising therapeutic option for treatment of acute SCI. Further, we extended our therapeutic studies with low dose (10 or 100 μg/kg) of E2 to chronic SCI rats and performed experiments to determine tissue integrity, reactive gliosis, status of blood flow, angiogenesis, expression of angiogenic factors, and axonal degeneration, and also locomotor function (Samantaray et al., 2016b). Results showed huge reduction in reactive gliosis, attenuation of damage to axons and myelin, protection of CNS cells, increases in expression of angiogenic factors and microvessel growth, and also significant improvement in locomotor function in chronic SCI animals due to E2 treatment at low dose when we compared them with the vehicle-treated chronic SCI rats. Thus, a low dose of E2 has significant therapeutic values in the treatment of chronic SCI as well. We think that more preclinical studies with E2 at a physiologically relevant low dose will be conducted to clearly establish the functional neuroprotection without side effects not only in acute SCI but also in chronic SCI animals before any clinical trials of E2 in SCI in humans.

The non-physiologically high dose of E2 may cause side effects such as feminization and tumorigenesis, which are impediments in clinical translation of E2 therapy in SCI in humans. Combination of therapeutic agents may be the future treatment strategy for functional repair and regeneration in SCI (Tohda and Kuboyama, 2011). Combination of

low doses of E2 and another therapeutic agent needs to be explored to increase the therapeutic outcomes of E2 therapy in SCI. Combination therapy should make it easy to use two different drugs at very low doses for additive effects or even synergistic effects, target diverse detrimental factors or pathophysiological pathways, discourage expression and encourage degradation of harmful molecules, and improve overall therapeutic outcomes in SCI. For example, we explored enhancement of therapeutic efficacy of the combination of miR-7-1 and E2 for protection in ventral spinal cord 4.1 (VSC4.1) motoneurons in the cell culture model of SCI (Chakrabarti et al, 2014b). We tested whether combination of miR-7-1 and E2 was more effective than either agent alone in preventing apoptosis in the calcium ionophore (CI)-insulted VSC4.1 motoneurons. Results showed that overexpression of miR-7-1 down-regulated L-type Ca^{2+} channel protein alpha 1C (CPα1C) but upregulated p-Akt (active) to trigger cell survival signaling and thus maximally increased efficacy of E2 for down-regulation of pro-apoptotic Bax protein and upregulation of anti-apoptotic Bcl-2 protein. We also studied whole cell membrane potential and mitochondrial membrane potential and found that miR-7-1 highly enhanced E2 therapeutic effects to promote functional neuroprotection in VSC4.1 motoneurons. Our data implicated that miR-7-1 could potentiate efficacy of E2 for improvement of locomotor function in animal model of SCI. However, this prediction needs to be validated experimentally in the near future.

We still do not have a magic bullet that can be readily used in the successful treatment or cure of SCI in humans. However, a number of preclinical studies from our and other laboratories so far strongly indicate that the multi-active E2 is a potential therapeutic candidate for treatment of SCI in humans. It should be clearly emphasized that E2 at 10 μg/kg is still not physiological and thus E2 therapy needs to be tried even at a lower dose in any future studies to avoid undesirable effects in both female and male SCI animals. We are currently conducting studies with further lower doses of E2 and finding that E2 at 5 μg/kg is also efficacious in inhibiting progressive pathogenesis and promoting locomotor function in SCI animals. Combination of perfectly physiological low dose of E2 with one more appropriate therapeutic agent should be an innovative avenue to enhance therapeutic values of E2 in preclinical models of SCI. It is highly likely that future research for treatment of SCI in animals will continue to focus on finding the lowest and most effective doses of E2 and selective enhancer of E2 efficacy in combination for achieving the ideal balance in therapeutic outcomes and side effects. Ideally, an estrogen receptor agonist (synthetic or plant-derived natural product) should have distinct mode of action in providing functional neuroprotection and no side effects in SCI animals. Novel synthetic or natural estrogen receptor agonist may show better pharmacology, more advantages, less disadvantages, and more functional neuroprotection in SCI. Therefore, future studies should also attempt to explore efficacies of the novel synthetic or plant-derived natural estrogenic molecules in improving overall outcomes in the treatment of SCI.

This work was supported in part by the grants from the South Carolina Spinal Cord Injury Research Fund (SC SCIRF-2015-I-01, Columbia, SC, USA) and the United Soybean Board (USB, Chesterfield, MO, USA) to SKR.

Swapan K. Ray*, Supriti Samntaray, Naren L. Banik

Department of Pathology, Microbiology, and Immunology, University of South Carolina School of Medicine, Columbia, SC, USA (Ray SK)
Department of Neurosciences, Medical University of South Carolina, Charleston, SC, USA (Samntaray S)
Department of Neurology and Neurosurgery, Medical University of South Carolina, Charleston, SC, USA (Banik NL)
Ralph H. Johnson Veterans Administration Medical Center, Charleston, SC, USA (Banik NL)

***Correspondence to:** Swapan K. Ray, Ph.D., swapan.ray@uscmed.sc.edu.*
***Accepted:** 2016-08-25*
***orcid:** 0000-0003-1734-542X (Swapan K. Ray)*
***doi:** 10.4103/1673-5374.191212*
***How to cite this article:** Ray SK, Samntaray S, Banik NL (2016) Future directions for using estrogen receptor agonists in the treatment of acute and chronic spinal cord injury. Neural Regen Res 11(9):1418-1419.*

References

Benyi E, Kieler H, Linder M, Ritzén M, Carlstedt-Duke J, Tuvemo T, Westphal O, Sävendahl L (2014) Risks of malignant and non-malignant tumours in tall women treated with high-dose oestrogen during adolescence. Horm Res Paediatr 82:89-96.

Chakrabarti M, Banik NL, Ray SK (2014b) miR-7-1 potentiated estrogen receptor agonists for functional neuroprotection in VSC4.1 motoneurons. Neuroscience 256:322-333.

Chakrabarti M, Das A, Samantaray S, Smith JA, Banik NL, Haque A, Ray SK (2016) Molecular mechanisms of estrogen for neuroprotection in spinal cord injury and traumatic brain injury. Rev Neurosci 27:271-281.

Chakrabarti M, Haque A, Banik NL, Nagarkatti P, Nagarkatti M, Ray SK (2014a) Estrogen receptor agonists for attenuation of neuroinflammation and neurodegeneration. Brain Res Bull 109:22-31.

Das A, Smith JA, Gibson C, Varma AK, Ray SK, Banik NL (2011) Estrogen receptor agonists and estrogen attenuate TNF-α-induced apoptosis in VSC4.1 motoneurons. J Endocrinol 208:171-182.

Farooque M, Suo Z, Arnold PM, Wulser MJ, Chou CT, Vancura RW, Fowler S, Festoff BW (2006) Gender-related differences in recovery of locomotor function after spinal cord injury in mice. Spinal Cord 44:182-187.

Ray SK, Samantaray S, Smith JA, Matzelle DD, Das A, Banik NL (2011) Inhibition of cysteine proteases in acute and chronic spinal cord injury. Neurotherapeutics 8:180-186.

Samantaray S, Das A, Matzelle DC, Yu SP, Wei L, Varma A, Ray SK, Banik NL (2016a) Administration of low dose estrogen attenuates gliosis and protects neurons in acute spinal cord injury in rats. J Neurochem 136:1064-1073.

Samantaray S, Das A, Matzelle DC, Yu SP, Wei L, Varma A, Ray SK, Banik NL (2016b) Administration of low dose estrogen attenuates persistent inflammation, promotes angiogenesis, and improves locomotor function following chronic spinal cord injury in rats. J Neurochem 137:604-617.

Tohda C, Kuboyama T (2011) Current and future therapeutic strategies for functional repair of spinal cord injury. Pharmacol Ther 132:57-71.