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Research Report

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

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ABSTRACT

17β-Estradiol is a multi-active steroid that imparts neuroprotection via diverse mechanisms of action. However, its role as a neuroprotective agent after spinal cord injury (SCI), or the involvement of the estrogen receptor-alpha (ER-α) in locomotor recovery, is still a subject of much debate. In this study, we evaluated the effects of estradiol and of Tamoxifen (an estrogen receptor mixed agonist/antagonist) on locomotor recovery following SCI. To control estradiol cyclical variability, ovariectomized female rats received empty or estradiol filled implants, prior to a moderate contusion to the spinal cord. Estradiol improved locomotor function at 7, 14, 21, and 28 days post injury (DPI), when compared to control groups (measured with the BBB open field test). This effect was ER-α mediated, because functional recovery was blocked with an ER-α antagonist. We also observed that ER-α was up-regulated after SCI. Long-term treatment (28 DPI) with estradiol and Tamoxifen reduced the extent of the lesion cavity, an effect also mediated by ER-α. The antioxidant effects of estradiol were seen acutely at 2 DPI but not at 28 DPI, and this acute effect was not receptor mediated. Rats treated with Tamoxifen recovered some locomotor activity at 21 and 28 DPI, which could be related to the antioxidant protection seen at these time points. These results show that estradiol improves functional outcome, and these protective effects are mediated by the ER-α dependent and independent-mechanisms. Tamoxifen's effects during late stages of SCI support the use of this drug as a long-term alternative treatment for this condition.

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1. Introduction

The estimated number of Americans that suffer from spinal cord injury (SCI) is 270,000 persons, with 12,000 new cases reported annually (National Spinal Cord Statistical Center, 2012). The main problem with SCI is that a cascade of independent events are initiated after the trauma; therefore, a combination therapy is required to better approach the progressive stages of this illness (Oudega et al., 2012). The devastating effects of SCI are associated with its immediate and secondary effects that result in cell degeneration at the trauma site and surrounding areas. Immediate damage that results from the direct physical impact includes hemorrhagic necrosis, excitotoxicity and the production of free radicals by the injured tissue (Bramlet and Dietrich, 2007). The secondary damage is a consequence mainly of an inflammatory response which involves apoptotic cell death, cyst formation and development of scar tissue (Hulsebosch, 2002). Therefore, a therapeutic strategy that addresses both stages is essential to target the condition and improve the outcome of SCI patients.

One of the clinical approaches to reduce neural damage after SCI is to curtail the inflammatory response by applying pharmacological doses of methylprednisolone. Although this treatment has been successful to inhibit secondary injury in animal models, it has controversial and questionable conclusions in clinical studies (Bydon et al., 2013). Development of multi-active compounds that target and/or block several of the detrimental cellular events triggered by the injury to the spinal cord are under intensive research.

Recent studies have explored the use of steroid hormones, like estradiol, which increase the viability of cells from the nervous system after a traumatic insult. In vitro and in vivo evidence shows that estradiol confers neuroprotection in different CNS pathologies and traumatic conditions including Alzheimer's disease, ischemia/stroke, and traumatic brain injury (Amtul et al., 2010; Dhandapani and Brann, 2002; Dubal et al., 2006; Etgen et al., 2011; Rau et al., 2003; Soustiel et al., 2005). One of the mechanisms by which estradiol confers neuroprotection is by reducing apoptosis (Chaovipoch et al., 2006; Sribnick et al., 2006a) and inducing activation of anti-apoptotic, neurotrophic, and regeneration associated genes (Scott et al., 2012; Segarra and Lee, 2004). In addition, its steroidal structure (phenol hydroxyl ring) confers anti-inflammatory and antioxidant properties, reducing cellular toxicity and death (Behl et al., 1997; Sugioka et al., 1987; Winterle et al., 2001).

The role of estradiol on locomotor recovery after SCI is still controversial. Swartz et al., 2007 showed that exposure to estradiol at low (28.2 pg/mL) or high (72 pg/mL) doses did not improve locomotor recovery in injured female rats. Baker & Haggs in 2005 concluded that the level of estradiol at different stages of the estrous cycle did not affect the functional outcome after SCI. In contrast, Yune et al., 2004 demonstrated that injecting 17β-estradiol before or immediately after SCI improved locomotor function and reduced the lesion size. In addition, Sribnick et al. (2005, 2010) showed that injecting a supraphysiological dose of estradiol immediately and 24 h after SCI reduced astrogliosis, reduced inflammation and decreased the extent of myelin loss by 2 days post-injury, an effect that persisted for 6 weeks after injury. To address these discrepancies, this study evaluated the effect of infusing constantly high physiological levels of estradiol to female rats before receiving a moderate contusion to the cord. Although this strategy could not be used in clinical practice, pretreatment of ovariectomized rats with estradiol controls the hormone's cyclical variability. Moreover, the continuous infusion of a high dose of estradiol, instead of a single application, should increase the availability of this neuroprotective agent and might further stimulate the body's neuroprotective response after SCI.

Neuroprotective effects of selective estrogen receptor modulators (SERMs) have also been reported (DonCarlos et al., 2009), without the complications that estradiol may generate, like the mitogenic effect on uterine and breast tissue. SERMs are compounds that interact with the estrogen receptors, producing estrogenic or antiestrogenic effects depending on the target tissue. Tamoxifen (TAM) is a SERM commonly used for the treatment of cancer in patients with tumors that test positive for the estrogen receptor, due to its antagonistic activity. Moreover, Tamoxifen exerts neuroprotection in amyotrophic lateral sclerosis, (Traynor et al., 2006), in ischemic brain injury (Dhandapani and Brann, 2002; Kimelberg et al., 2003; Mehta et al., 2003; Zhang et al., 2005) and acutely after SCI, when analyzed at 6 h (Ismailoglu et al., 2010), 7 days (Tian et al., 2009) or 35 days post-injury (Guptarak et al., 2014). The hypothesis to be tested is that TAM exerts long-term neuroprotective effects in these cells after physical trauma to the adult spinal cord.

The current study assessed the neuroprotective effects of estradiol and Tamoxifen pretreatment on recovery after SCI. The following parameters were measured: (1) recovery of hindlimb motor function (assessed using the BBB open field test), (2) amount of spared tissue (determined with Luxol staining), and (3) oxidative stress (measured by superoxide production). Insight into the mechanism of action was obtained by blocking the estrogen receptor (ER)-α with the selective antagonist MPP Dihydrochloride (MPP) and investigating the temporal pattern of ER-α expression after trauma. Therefore, the hypothesis tested was that pretreatment followed by continuous infusion of physiological high levels of estradiol will exert acute and chronic neuroprotective effects in a contused spinal cord model and that ER mediates this effect.

2. Results

2.1. *Estradiol plasma levels after Silastic implants*

Rats with Silastic implants containing 3 mg 17β-Estradiol-benzoate show that estradiol levels peaked at day 7, the time point when the SCI was performed (Table 1). Mean plasma levels of estradiol at the time of SCI were approximately 154 pg/mL and decrease gradually over time reaching 86.3 pg/mL by week 4 after Silastic tube implant. Control group (Silastic implant empty) had a mean low level of approximately 8 pg/mL (see Table 1) throughout the 4 weeks. In addition to plasma levels, verification of estradiol release