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17β-estradiol and tamoxifen protect mice from manganese-induced dopaminergic neurotoxicity

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ABSTRACT

Chronic exposure to manganese (Mn) causes neurotoxicity, referred to as manganism, with common clinical features of parkinsonism. 17β-estradiol (E2) and tamoxifen (TX), a selective estrogen receptor modulator (SERM), afford neuroprotection in several neurological disorders, including Parkinson's disease (PD). In the present study, we tested if E2 and TX attenuate Mn-induced neurotoxicity in mice, assessing motor deficit and dopaminergic neurodegeneration. We implanted E2 and TX pellets in the back of the neck of ovariectomized C57BL/6 mice two weeks prior to a single injection of Mn into the striatum. One week later, we assessed locomotor activity and molecular mechanisms by immunohistochemistry, real-time quantitative PCR, western blot and enzymatic biochemical analyses. The results showed that both E2 and TX attenuated Mn-induced motor deficits and reversed the Mn-induced loss of dopaminergic neurons in the substantia nigra. At the molecular level, E2 and TX reversed the Mn-induced decrease of (1) glutamate aspartate transporter (GLAST) and glutamate transporter 1 (GLT-1) mRNA and protein levels; (2) transforming growth factor-α (TGF-α) and estrogen receptor-α (ER-α) protein levels; and (3) catalase (CAT) activity and glutathione (GSH) levels, and Mn-increased (1) malondialdehyde (MDA) levels and (2) the Bax/Bcl-2 ratio. These results indicate that E2 and TX afford protection against Mn-induced neurotoxicity by reversing Mn-reduced GLT1/GLAST as well as Mn-induced oxidative stress. Our findings may offer estrogenic agents as potential candidates for the development of therapeutics to treat Mn-induced neurotoxicity.

1. Introduction

Manganese (Mn) is an essential element for development, metabolism and antioxidant systems (Keen et al., 1999). However, chronic exposure to excessive Mn levels leads to its accumulation in the globus pallidus and other basal ganglia nuclei, culminating in a neurological disorder referred to as manganism (Barbeau, 1984; Calne et al., 1994). Manganism exhibits clinical symptoms analogous to Parkinson's disease (PD), characterized by motor impairment, psychiatric and cognitive deficits (Calne et al., 1994; Guilarte, 2010). The symptoms of manganism and PD are associated with loss of dopaminergic neurons in the substantia nigra, resulting in dopaminergic dysregulation-related pathological symptoms.

Although Mn-induced pathological symptoms are well established, cellular/molecular mechanisms of Mn neurotoxicity are not completely understood. Among several mechanisms proposed for Mn-induced toxicity, oxidative stress and glutamate-mediated excitotoxicity appear critical and widely supported by numerous studies (Lee et al., 2017; Martinez-Finley et al., 2013), providing a putative target for developing therapeutics for Mn-induced neurotoxicity. Notably, excitotoxic neuronal death is associated with various neurodegenerative diseases, including multiple sclerosis, Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), PD, and manganism (Sheldon and Robinson, 2007). Glutamate transporters play a crucial role in maintaining optimal glutamate levels in the synaptic cleft to mitigate excitotoxic neuronal injury (Gegelashvili and Schousboe, 1997).

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Among the five identified human excitatory amino acid transporters (EAATs), two main glutamate transporters responsible for preventing excitotoxic neuronal injury are EAAT1 and EAAT2, referred to as glutamate-aspartate transporter (GLAST) and glutamate transporter-1 (GLT-1) in rodents, respectively. Both transporters are predominantly expressed in astrocytes, accounting for >90% of synaptic glutamate clearance (Danbolt, 2001; Kim et al., 2011). Mn decreases expression as well as function of the astrocytic glutamate transporters, GLAST and GLT-1 (Erikson and Aschner, 2002; Gillessen et al., 2002; Karki et al., 2014a), resulting in glutamate accumulation in the synaptic clefts and the ensuing excitotoxic neuronal injury (Sidoryk-Wegrzynowicz et al., 2009).

The female sex hormone 17β-estradiol (E2) exerts neuroprotection in various neurological disease models, including PD (Ramirez et al., 2003). Notably, the incidence of PD is 1.5 times greater in men than those in women, suggesting that E2 might contribute to the lower incidence of the disease (Natrajan and Gambrell, 2002; Wooten et al., 2004). Several selective estrogen receptor modulators (SERMs) such as tamoxifen (TX) which has agonistic or antagonistic effects on the estrogen receptors (ERs) depending on tissue types, have shown to exert neuroprotection. Numerous clinical and animal studies have demonstrated the neuroprotective effects of E2 and SERMs including TX in various neurodegenerative diseases, including PD models (Bourque et al., 2009; Cyr et al., 2002; Dluzen et al., 2001; Kuo et al., 2003).

Anti-oxidative effects appear to be involved in E2- and TX-induced neuroprotection in several animal models of neurological disorders with ER-dependent and −independent mechanisms (Abdelhamid et al., 2011; Bourque et al., 2009; D'Astous et al., 2004; Wang et al., 2015; Zhang et al., 2007; Zou et al., 2015). E2 ameliorated light-induced retinal damage by antioxidant mechanism (Wang et al., 2015), as well as enhancing phase-2 antioxidant enzyme expression including catalase and superoxide dismutase by ER-dependent mechanisms in rats (Zhu et al., 2015). TX protected focal cerebral ischemia in rats via ER-independent antioxidant effects (Zhang et al., 2007). We have previously reported that E2 and TX attenuated Mn-induced oxidative stress in *in vitro* primary astrocytes (Lee et al., 2009).

The cell-type specificity of E2- and TX-neuroprotection has not been completely understood, but astrocytes appear to play a critical role in mediating this effect (Spence et al., 2011). E2 has shown to reduce the cortical lesion size induced by ibotenate, a glutamate analogue that activates *N*-methyl-D-aspartate (NMDA) in neonatal rats by astrocytic mechanism (Pansiot et al., 2016). Moreover, our previous study demonstrated that E2, TX and raloxifene (RX) attenuated Mn-induced impairment of astrocytic glutamate transporters (Lee et al., 2012, 2009). Given that astrocytic glutamate transporters is a critical regulator of synaptic glutamate levels, modulation of these transporters could be involved in E2/SERMs-induced protective effects in animal models of excitotoxic brain injury (Armagan et al., 2009; Ciriza et al., 2004; Mendelowitsch et al., 2001; O'Neill et al., 2004; Zhang et al., 2009).

In the present study, we determined if E2 and TX protect against Mn-induced neurotoxicity *in vivo*. We implanted E2 and TX pellets in the back of ovariectomized C57BL/6 mice two weeks prior to a single injection of Mn into the striatum lasting a week, followed by assessing locomotor activity and dopaminergic neuronal damage along with relevant molecular/biochemical assays. Our results showed that both E2 and TX afforded neuroprotection against Mn-induced dopaminergic neurotoxicity by reversing Mn-reduced locomotor activity, tyrosine hydroxylase (TH) mRNA/protein levels, astrocytic GLT-1/GLAST mRNA/protein levels, and antioxidant markers such as glutathione (GSH) and catalase (CAT).

2. Materials and methods

2.1. Experimental animals

All animal protocols were reviewed and approved by the Meharry Medical College Institutional Animal Care and Use Committee (Nashville, TN). The ovariectomized female C57BL/6 mice (12 weeks old; weight 18–20 g) were purchased from the Jackson Laboratory (Bar Harbor, ME). Animals were housed and maintained on a 12-h light/dark cycle at 25 ± 2 °C and 60–70% relative humidity with food and water available *ad libitum* in the Animal Care Facility of Meharry Medical College (Nashville, TN).

2.2. Chemicals and reagents

Manganese chloride ($MnCl_2$, Cat. # 244589) was obtained from Sigma-Aldrich (St. Louis, MO). Pellets of 17β-estradiol (E2, Cat. # E-121) and its placebo (Cat. # C-111), and tamoxifen citrate (TX, Cat. # E-351) and its placebo (Cat. # C-111) were obtained from Innovative Research of America (Sarasota, FL). EAAT1/GLAST (ab416) and EAAT2/GLT-1 (ab41621) antibodies were obtained from Abcam (Cambridge, MA). Antibodies for TH (sc-25269), transforming growth factor-α (TGF-α, sc-36), ER-α (sc-542), catalase (CAT, sc-271803), Bax (sc-7480), Bcl-2 (sc-7382) and β-actin (sc-47778) were acquired from Santa Cruz Biotechnology (Santa Cruz, CA). Rabbit anti-mouse IgG H&L horseradish peroxidase (HRP)-conjugated (ab6728), Goat anti-rabbit IgG H&L HRP-conjugated (ab97051) and donkey anti-goat (ab97110) IgG H&L horseradish peroxidase (HRP)-conjugated and goat anti-mouse IgG Alexa Fluor® 568 (ab150119) secondary antibodies were obtained from Abcam. CAT activity colorimetric/fluorometric kit (Cat. # K773-100) and glutathione (GSH) fluorometric assay kit (Cat. # K251-100) were obtained from BioVision Inc. (Milpitas, CA). Malondialdehyde (MDA)/lipid peroxidation/TBARS assay kit (Cat. # 10009055) was obtained from Cayman Chemical (Ann Arbor, MI).

2.3. Experimental procedure

Ovariectomized C57BL/6 mice were randomly assigned to one of the following eight groups (n = 7–8 per group): (1) Control (sham), (2) E2 placebo, (3) TX placebo, (4) Mn, (5) E2, (6) TX, (7) E2 + Mn and (8) TX + Mn. E2 pellets (0.25 mg/pellet, 21-d release) and TX pellets (5 mg/pellet, 21-d release) along with their placebo pellets were used (Sasayama et al., 2017). All pellets (Innovative Research of America, Sarasota, FL, USA) were inserted in chloral hydrate-anesthetized (400 mg/kg i.p.) mice subcutaneously into a 0.5 cm incision made along the loose skin of the mouse's neck (Pan et al., 1994). The pellets were inserted with tweezers into a small pocket, which was formed by bluntly dissecting caudolaterally (Subramanian et al., 2017). The incision and the closed suture (made with a wound clips) were performed under aseptic techniques to minimize the risk of infection. E2 and TX were administered as pellets for two weeks (Strom et al., 2008). $MnCl_2$ (0.4 μl of 1 μmol/μl of $MnCl_2$ in H_2O) was administered into the right striatum by stereotaxic injection in each mouse in groups of Mn, Mn plus E2, Mn plus TX one week before animals sacrificed (Brouillet et al., 1993; Sloot et al., 1994; Zhao et al., 2009). Chloral hydrate-anesthetized (400 mg/kg, i.p.) mice from each treatment group were placed in a stereotaxic frame with a nose bar. For the stereotaxic injection, a hole was drilled and the following stereotaxic coordinates were aimed: anteroposterior (bregma) = + 0.8 mm; mediolateral = + 1.9 mm; dorsoventral = 3.4 mm, corresponding similar studies for right striatum and Allen Brain Atlas (Sloot et al., 1994; Zhao et al., 2009).

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