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Review

Selective estrogen receptor modulators (SERMs) enhance neurogenesis and spine density following focal cerebral ischemia

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ABSTRACT

Selective estrogen receptor modulators (SERMs) have been reported to enhance synaptic plasticity and improve cognitive performance in adult rats. SERMs have also been shown to induce neuroprotection against cerebral ischemia and other CNS insults. In this study, we sought to determine whether acute regulation of neurogenesis and spine remodeling could be a novel mechanism associated with neuroprotection induced by SERMs following cerebral ischemia. Toward this end, ovariectomized adult female rats were either implanted with pellets of 17β-estradiol (estrogen) or tamoxifen, or injected with raloxifene. After one week, cerebral ischemia was induced by the transient middle-cerebral artery occlusion (MCAO) method. Bromodeoxyuridine (BrdU) was injected to label dividing cells in brain. We analyzed neurogenesis and spine density at day-1 and day-5 post MCAO. In agreement with earlier findings, we observed a robust induction of neurogenesis in the ipsilateral subventricular zone (SVZ) of both the intact as well as ovariectomized female rats following MCAO. Interestingly, neurogenesis in the ipsilateral SVZ following ischemia was significantly higher in estrogen and raloxifene-treated animals compared to placebo-treated rats. In contrast, this enhancing effect on neurogenesis was not observed in tamoxifen-treated rats. Finally, both SERMs, as well as estrogen significantly reversed the spine density loss observed in the ischemic cortex at day-5 post ischemia. Taken, together these results reveal a profound structural remodeling potential of SERMs in the brain following cerebral ischemia.

This article is part of a Special Issue entitled "Sex steroids and brain disorders".

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Abbreviations: BrdU, 5-bromo-2′-deoxyuridine; CNS, central nervous system; DCX, doublecortin; E2, 17β-estradiol; ER, estrogen receptor; MCAO, middle cerebral artery occlusion; NPCs, neural progenitor cells; PBS, phosphate buffered saline; SERM, selective estrogen receptor modulator; SVZ, subventricular zone; TTC, 3,5-triphenyltetrazolium chloride.

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1. Introduction

Selective estrogen receptors modulators (SERMs) are a class of compounds, which act as agonists or antagonists to estrogen receptors (ERs) depending on the tissue types. Two well-studied SERMs are tamoxifen and raloxifene; which were initially designed for the treatment of breast cancer and osteoporosis, respectively. However, a number of studies suggest that tamoxifen and raloxifene can have neuroprotective effects in vitro or in vivo against neurological insults or in neurological disorders [1–11]. Additionally, both tamoxifen and raloxifene have also been shown to enhance memory, cognition, and synaptic plasticity in various experimental studies [12,13]. However the mechanisms underlying the beneficial neural effects of SERMs are still unclear. Findings from our group and others suggest that tamoxifen can exert neuroprotection by regulating kinase signaling pathways, antioxidant enzymes, and mitochondrial reactive oxygen species generation [7,9]. In contrast, the mechanisms of raloxifene neuroprotection have not been studied in detail and are poorly understood. It is known that tamoxifen and raloxifene can recruit different coactivators or corepressors to the ER complex, which may account, in part, for the well known divergent effects of the SERMs in some tissues.

Under various experimental conditions, 17β-estradiol (E2) is known to prevent cell death, promote neuronal survival, and enhance synaptic plasticity in the brain [14–20]. In addition, a number of studies have shown that E2 can enhance neurogenesis, leading to production of new neurons in the affected cerebral hemisphere [21–23]. New neurons are produced from the neural progenitor cells (NPCs) residing along the lateral wall of subventricular zone (SVZ) in the forebrain and the dentate gyrus of hippocampus [24–26]. In the forebrain, a majority of NPCs from the SVZ have been shown to migrate toward the sites of injury, including the cortex and striatum [27–29]. The SVZ-derived NPCs differentiate into mature neurons at the sites of injury and form synapses with neighboring cells [30]. These findings suggest that estradiol plays an important trophic as well as neuroprotective role in the adult brain. However, findings from the Women' Health Initiative suggested that estrogens, under some circumstances, might actually increase the risk of neurodegeneration [31–33]. While this finding is controversial, it has increased the search for alternative estrogenic compounds that possess minimal negative side effects, while retaining beneficial neural effects.

In the current study, we sought to examine whether clinically relevant SERMs can regulate neurogenesis and spine density in the brain as mechanisms to reduce brain injury and enhance neural repair following focal cerebral ischemia in ovariectomized female rats. For comparison, the effects of estrogen were also examined. Since the SVZ can be a source of new neurons after focal cerebral ischemia [27–29], we examined the effect of clinically relevant doses of the SERMs, tamoxifen and raloxifene (as well as estrogen) on neurogenesis and spine density in the affected cerebral hemisphere following transient and permanent middle cerebral artery occlusion (MCAO) in ovariectomized female rats. The results suggest that estrogen and raloxifene enhance neurogenesis in the ipsilateral SVZ following MCAO. Tamoxifen, on the other hand had no significant effect on neurogenesis in the SVZ. Interestingly, all three compounds significantly reduced ischemia-induced spine density loss in the ipsilateral cortex following MCAO. These findings provide evidence for a potential role of estrogen and SERMs in cortical remodeling following ischemic brain injury.

2. Experimental procedures

2.1. Animals and drug treatment

All experiments were conducted in compliance with the National Institutes of Health guidelines for the care and use of experimental animals and were approved by the Institutional Animal Care and Use Committee. Sixty-day-old Holtzman Sprague Dawley female rats (Harlan, IN) were used for the study. The animals were housed in individual cages and water and rat chow was provided *ad libitum*. The animals were bilaterally ovariectomized and implanted subcutaneous (*sc*) in the mid-upper back region with pellets that contained placebo, E2 (0.025 mg which produces low diestrus [10–15 pg/ml] levels of E2) [34] or tamoxifen (15 mg pellets, which releases ~1 mg/kg/d of tamoxifen) [9]. In addition, an additional group of ovariectomized rats were injected intramuscularly with raloxifene at a daily dose of 10 mg/kg. One week later, all animals underwent surgery to induce cerebral ischemia as described below.

2.2. Induction of cerebral ischemia

Focal cerebral ischemia was induced using the transient middle cerebral artery occlusion (MCAO) method as described previously by our laboratory [9]. Briefly, rats were anesthetized with ketamine/xylazine (intramuscular, 60 mg/ml and 8 mg/ml, respectively). A thermal blanket was used to maintain body temperature at 37 °C. The skin of the neck was shaved and swabbed with betadine, and an incision was made directly on top of the right common carotid artery region. The fascia was then blunt dissected until the bifurcation of the external common carotid artery and internal common carotid artery was isolated. A small incision was made in the external common carotid artery and then a 4–0 monofilament