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17β-estradiol valerate regulates Protein Disulfide Isomerase, c-fos and Nrf2 gene expressions to provide ischemic neuroprotection in ovariectomized rat

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Background and purpose - Protein Disulfide Isomerase (PDI) is a multifunctional protein mainly located in the endoplasmic reticulum (ER). Circulating estrogen is positively associated with neuroprotection against ischemia in female rats. In the present study, we examined whether the effects of estrogen on the brain damage and on the transcriptional levels of PDI gene, known to play a role in antioxidant systems after transient focal ischemia in rat brain.

Methods - Female animals were ovariectomized, treated with 100μg/kg 17β-estradiol valerate (EV) or sesame oil treats, and subjected to 1-hour unilateral middle cerebral artery occlusion (MCAO) and 24 hours of reperfusion. At 24 hours after MCAO, the brains were cut into four 2-mm-thick slices and stained with 2% TTC and cresyl-violet. Total RNA was extracted using the acid guanidinium isothiocyanate-phenol-chloroform method using Tri-reagent. The expression of PDI, c-fos and Nrf2 mRNA were assessed by RT-PCR and *in situ* hybridization.

Results - Histopathological analysis revealed a significant decrease in infarct size in the ipsilateral brain on EV treated group compared with vehicle group. The greatest total infarct size was 71.4% in the vehicle group. Treatment with EV for 3 d prior to the induction of ischemia attenuated the total brain infarction (32.3%). The cresyl violet stain

and *in situ* hybridization showed significant increase the surviving neurons mRNA in the CA1 hippocampus treated EV group compared with vehicle group. Moreover, the levels of PDI and Nrf2 transcription was significant increased in the EV group versus untreated group ($P<0.05$).

Conclusion - This study therefore indicated that EV provide neuroprotection by preventing brain damage through the increased expression of genes encoding antioxidant proteins after transient focal cerebral ischemia. EV may be effective as neuroprotective agents at the cellular and molecular levels in the brain.

Keywords: *17β-estradiol valerate, ischemia, neuroprotection, protein disulfide isomerase, c-fos, Nrf2*