

Estradiol-Mediated Regulation of Inflammation During Stroke Injury in the Adult and Aging Brain

Candice M. Brown, Ph.D., Postdoctoral Fellow; Phyllis Wise, Ph.D., Professor & Provost, Department of Physiology and Biophysics, School of Medicine, University of Washington

Background: The focus of my research is to understand how 17 β -estradiol (E2) protects against brain injury associated with ischemic stroke. Women appear to be protected against stroke compared to men since studies show that premenopausal women have a lower incidence of stroke compared to age-matched males, but this protection is lost after the menopause. The biological basis of this protection is thought to result from the neuroprotective properties of E2. Several studies from our laboratory and others have shown that physiological levels of E2 exhibit profound neuroprotective properties following cerebral ischemia that are mediated through multiple molecular mechanisms. More recent studies also suggest that E2 possesses anti-inflammatory neuroprotective properties via suppression of inducible nitric oxide synthase (iNOS), an enzyme with a primarily deleterious role in the pathophysiology of cerebral ischemia. While previous work has investigated some of E2's potential molecular mechanisms of action, specifically relating to apoptosis, few studies have explored the unique relationship between E2 and inflammation during cerebral ischemia.

Objectives: My studies are focused on testing the hypothesis that physiological concentrations of E2 decrease post-ischemic inflammation associated with the brain and periphery and address the following critical questions:

- 1) How does E2 regulate inflammatory mechanisms promoting delayed cell death in the ischemic penumbra?
- 2) Does E2 inhibit the normal temporal evolution of delayed cell death in the ischemic penumbra, and
- 3) How do ER α and ER β regulate inflammatory responses in the ischemic penumbra?

Methods: To explore the neuroprotective properties of E2 during ischemic stroke, I utilize a permanent model of middle cerebral artery occlusion (MCAO) in C57BL/6 mice, iNOS knockout (iNOSKO) mice, and both estrogen receptor alpha and beta knockout (ER α KO and ER β KO) mice. Mice are subjected to MCAO after one week and killed 6, 12, 24, or 96 hours later. Infarct volumes are measured in 1-mm brain sections treated with 2% triphenyltetrazolium chloride to delineate the extent of injury. Plasma and brain cytokine levels are measured using a multiplex proteomic approach incorporating Luminex technology. In addition, both real time PCR and immunocytochemistry are used to analyze both spatial and temporal changes in gene and protein expression throughout the brain. By using MCAO as a model of ischemic stroke, answers to these questions will ultimately elucidate the molecular mechanisms underlying E2's role in brain injury as well as chronic neuroinflammatory conditions associated with aging.