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Research Report

High-dose estrogen treatment at reperfusion reduces lesion volume and accelerates recovery of sensorimotor function after experimental ischemic stroke

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ABSTRACT

Estrogens have previously been shown to protect the brain against acute ischemic insults, by potentially augmenting cerebrovascular function after ischemic stroke. The current study hypothesized that treatment with sustained release of high-dose 17β-estradiol (E2) at the time of reperfusion from middle cerebral artery occlusion (MCAO) in rats would attenuate reperfusion injury, augment post-stroke angiogenesis and cerebral blood flow, and attenuate lesion volume. Female Wistar rats underwent ovariectomy, followed two weeks later by transient, two-hour right MCAO (tMCAO) and treatment with E2 ($n=13$) or placebo (P; $n=12$) pellets starting at reperfusion. E2 treatment resulted in significantly smaller total lesion volume, smaller lesions within striatal and cortical brain regions, and less atrophy of the ipsilateral hemisphere after six weeks of recovery. E2-treated animals exhibited accelerated recovery of contralateral forelimb sensorimotor function in the cylinder test. Magnetic resonance imaging (MRI) showed that E2 treatment reduced the formation of lesion cysts, decreased lesion volume, and increased lesional cerebral blood flow (CBF). K^{trans}, a measure of vascular permeability, was increased in the lesions. This finding, which represents lesion neovascularization, was not altered by E2 treatment. Ischemic stroke-related angiogenesis and vessel formation was confirmed with immunolabeling of brain tissue and was not altered with E2 treatment. In summary, E2 treatment

Abbreviations: E2, 17β-estradiol; P, Placebo; MCAO, Middle cerebral artery occlusion; TMCAO, Transient MCAO; MRI, Magnetic resonance imaging; CBF, Cerebral blood flow; CBV, Cerebral blood volume; IV-tPA, Intravenous administration of tissue plasminogen activator; ROI, Region of interest

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administered immediately following reperfusion significantly reduced lesion size, cyst formation, and brain atrophy while improving lesional CBF and accelerating recovery of functional deficits in a rat model of ischemic stroke.

1. Introduction

The role of estrogen in protection against ischemic stroke began with epidemiological data demonstrating that premenopausal women have a lower incidence of stroke and cardiovascular disease compared to men of the same age (Petrea et al., 2009). Removal of female sex hormones by ovariectomy in animal models of ischemic stroke, such as the MCAO, resulted in an exacerbation of injury that could be reversed by exogenous administration of estrogen (Alkayed et al., 1998; Simpkins et al., 1997). However, clinical trials showed a lack of benefit of hormone replacement therapy in preventing primary or secondary incidence of stroke (Rossouw, 2002; Viscoli et al., 2001), severely hampering the potential for estrogen to be used as a prophylactic treatment for ischemic stroke.

The administration of estrogen during or after an ischemic stroke may still be able to provide neuroprotection, alter repair, and restore neurological function. Simpkins et al. (1997) showed that high dose E2 at reperfusion could increase survival to one week after stroke and reduce lesion size. While high and low doses of E2 had beneficial effects when administered prior to permanent MCAO (pMCAO), only high physiological levels were able to perform the same function when administered at the onset of ischemia (Dubal et al., 1998). Yang et al. (2000) established the window of efficacy for supraphysiologic E2 administration to be ≤ 3 h after the onset of pMCAO, and confirmed that low endogenous levels offered no protection. Importantly, they also showed that early supraphysiologic E2 treatment could increase ipsilateral CBF 24 h after experimental stroke, suggesting a potential cerebrovascular component for E2-mediated neuroprotection.

The ability of estrogen to alter CBF was further investigated with a single intravenous injection of high-dose Premarin after tMCAO, which reduced hypoperfusion and increased CBF during the acute period of reperfusion, resulting in a reduction of lesion volume (McCullough et al., 2001). The fast onset and transient nature of this effect suggested a non-genomic mechanism of CBF regulation. Liu et al. (2002) provided additional evidence of a non-genomic mechanism with the administration of a non-estrogen receptor binding E2 derivative. The derivative increased ipsilateral and contralateral CBF during reperfusion while also retaining antioxidant properties similar to E2. Although it is difficult to attribute decreases in lesion size to any one specific mechanism, the changes in cerebrovascular function after E2 treatment seem to be associated with improved outcomes after experimental ischemic stroke.

We have previously shown that administration of E2 one week prior to tMCAO in ovariectomized female rats enhanced post-stroke angiogenesis without significantly altering lesion size, which also resulted in an improvement in contralateral forelimb function three weeks after ischemic stroke (Ardelt et al., 2012). MRI demonstrated improvement in CBF during the fourth week of recovery with E2 pre-treatment. Estrogen may have enhanced angiogenesis by increasing the expression of angiopoietin-1 (Ardelt et al., 2005) and promoting endothelial cell survival (Guo et al., 2010) after ischemic stroke. The collective actions of E2 on cerebrovascular function represent important and novel targets for enhancing current treatment strategies.

The current study was designed to understand if E2 treatment during reperfusion could alter long-term components of cerebrovascular function, lesion volume, and post-stroke repair. For this, ovariectomized female Wistar rats underwent a two-hour right MCAO followed by reperfusion and E2 or placebo pellet implantation (Fig. 1). Animals were randomly assigned to the treatment groups, and all analyses were performed in a blinded fashion. Forelimb sensorimotor impairment was assessed weekly for six weeks after experimental stroke, at which time animals were euthanized and histological sections were analyzed for lesion volume and biomarkers of post-stroke injury and repair. A subset of animals underwent MRI with contrast administration for evaluation of CBF and vascular permeability, as well as measurement of lesion volume four weeks after experimental stroke. We hypothesized that administration of high-dose E2 at reperfusion would reduce lesion volume, improve cerebrovascular function, and enhance recovery during long-term survival after transient cerebral ischemia.

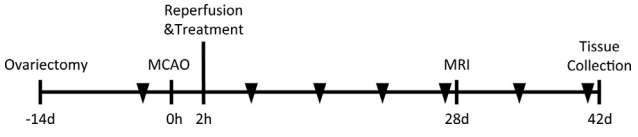

Fig. 1 – Experimental outline. Female Wistar rats were ovariectomized fourteen days prior to experimental stroke. Reperfusion and treatment (placebo or 17β-estradiol pellet) took place after two hours of middle cerebral artery occlusion (MCAO). Representative rats in both groups underwent MRI analysis of lesion volume and cerebrovascular function after four weeks of recovery. Brain tissue samples were collected for histology after six weeks of recovery. Triangles represent weekly behavioral assessment of forelimb function with the cylinder test. All animals were randomly assigned to the treatment groups and all analyses were performed in a blinded fashion.