2nd International Conference on
Cardiovascular Diseases & Therapeutics
August 12-13, 2019 | Amsterdam, Netherlands

The Role of Estrone in Endothelial Progenitor Cell Number and Function

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Microvascular dysfunction is a key component of cardiovascular disease and estrogens, particularly estradiol, have been shown to be vascular protective. Endothelial progenitor cells (EPCs) are a stem cell population that supports the integrity of the microvasculature and show increased proliferation in response to estradiol exposure. The plasma concentration of EPCs negatively correlates with incidence of stroke and coronary artery disease. Another estrogen, estrone, has been understudied due to its weak binding at nuclear estrogen receptors ERa and ERb. However, recent evidence suggests that estrone is biologically active through a G protein coupled estrogen receptor, GPER. In some vascular cell populations, estrone independently increases proliferation and modulates the proliferative effects of estradiol. In pre-menopausal women with high estradiol/estrone ratio, EPCs are present in sufficient numbers to enhance angiogenesis and repair vessels at sites of damage. After menopause when the estradiol/estrone ratio is low, EPC number is decreased, the microvasculature is compromised, and CVD risk increases. In the treatment of symptoms of menopause, we use a combination estrogen therapy which is largely estrone based. Using this therapy, estradiol levels are restored pharmacologically, but the estradiol/estrone ratio remains low. During treatment, EPC numbers remain low and cardiovascular risk remains high.

Here we investigate the role of estrone in EPC proliferation via in vitro exposure studies using EPCs from human female blood samples and EPCs isolated from the bone marrow of Sprague Dawley Rats. We also assess how estrone, estradiol and combination exposures effect the ability of EPCs to enhance endothelial cell tube formation (vasculogenesis in vitro). Human and rat EPCs were defined as cells derived from the mononuclear cell fraction that after 10–14 days of expansion/selection in culture adhere to fibronectin, display typical endothelial cell (EC) morphology and are positive for the EPC markers VEGFR2, CD133, cKIT, CD34, and Dil-ac-LDL uptake. After sample collection (human peripheral blood or rat bone marrow), the mononuclear cell layer was isolated using density centrifugation. The mononuclear cell layer was then plated in endothelial growth media for 14 days. After 14 days the EPCs cells were used for expression, proliferation and tube formation assays. The proliferation assays were performed over a 24 hour exposure to 1 & 2 nmol estrone, 1 & 2 nmol estradiol and 1:1 (1nmol) estrone:estradiol. In our tube formation assay, EPCs were exposed to estrone, estradiol and 1:1 estrone:estradiol for 24 hours and then added to an endothelial cell tube formation assay. We assessed GPER expression via qPCR and immunocytochemistry on EPCs without hormone treatment.

In vitro EPC proliferation was enhanced by both estrone and estradiol in a dose dependent manner. When estrone and estradiol were applied in a one to one ratio, resulting EPC proliferation was below that of control. GPER is expressed on both human and rat EPCs. Further studies will use a rat model to investigate physiological effects of estrone and GPER agonism on EPC ability to enhance angiogenesis in vivo.