74. NOVEL ERα LIGANDS, PPT AND R,R-THC, PROMOTE ANGIOGENESIS IN HUMAN MYOMETRIAL MICROVASCULAR ENDOTHELIAL CELLS

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Angiogenesis is the growth of new blood vessels from pre-existing vessels and involves proliferation of microvascular endothelial cells (MEC). VEGF is a major promoter of angiogenesis and mediates angiogenic effects through interaction with VEGF receptor-2 (VEGF-R2). We have demonstrated that MEC derived from human myometrium (MMEC) constitutively express estrogen receptor-β (ERβ), while ERα varies between subjects and is only expressed in approximately 60% of MEC isolates[1]. 17β-estradiol (E) upregulates VEGF-R2 and promotes MEC proliferation in the ERα-expressing isolates, but not in ERα negative MMEC[2]. The aim of the present study was to determine whether ERα mediates upregulation of VEGF-R2 and the angiogenic effects of E in ERα-expressing adult human MEC using the novel ERα-selective ligands, PPT and R,R-THC[3]. Myometrial MEC were isolated from hysterectomy tissue obtained from ovulating women, cultured and used between passages 1-3 (purity >98% CD31+ cells)[4]. ERα and VEGF-R2 expression were measured by flow cytometry using an ERα antibody and biotin-rhVEGF$_{165}$ binding respectively[2]. MEC proliferation was determined by MTS bioassay[2]. We first tested the activity of PPT and R,R-THC on a breast tumour cell line known to express wildtype ERα (MCF-7) and demonstrated that both ligands significantly increased proliferation in a similar manner to E, an effect blocked by the nonspecific ER antagonist, ICI 182,780. Neither PPT or R,R-THC stimulated proliferation of the ERα negative cell line MDA-DB-453. In ERα+ MMEC, PPT and E increased VEGF binding in a dose-dependent manner, but had no effect on ERα negative MMEC samples. PPT, R,R-THC and E significantly augmented VEGF-induced MMEC proliferation in ERα positive MMEC ($P< 0.05$) but not ERα negative MMEC. These data confirm that the angiogenic effects of E on MMEC are due to upregulation of VEGF-R2 and are mediated by ERα rather than ERβ.

(1) Gargett CE *et al.* (2002) *Mol. Hum. Reprod.* **8**: 770–775. (2) Gargett CE *et al.* (2002) *J. Clin. Endocrinol. Metab.* **87**: 4341–4349. (3) Kraichely DM *et al.* (2000) *Endocrinol.* **141**: 3534–3545. (4) Gargett CE *et al.* (2000) *Hum. Reprod.* **15**: 293–301.

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