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YC-1 reduces placental sFlt-1 and soluble endoglin production and decreases endothelial dysfunction: A possible therapeutic for preeclampsia

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ABSTRACT

Preeclampsia is a serious complication of pregnancy with no medical treatment. It is caused by intermittent placental hypoxia and release of sFlt-1 and soluble endoglin, leading to wide spread maternal endothelial dysfunction and multisystem organ injury. YC-1 is a guanylyl cyclase activator and HIF1α inhibitor developed for use in hypertension and atherosclerosis. We examined whether YC-1 reduces sFlt-1 and sENG secretion and reverses endothelial dysfunction in primary human tissues. YC-1 significantly reduced sFlt-1 and sENG secretion from human umbilical vein endothelial cells, purified primary trophoblast cells and placental explants taken from patients with preterm preeclampsia. This was concordant with reduced HIF1α expression. YC-1 also reversed TNFα induced endothelial dysfunction, including reduced vascular cell adhesion molecule 1 expression and monocyte adhesion to primary endothelial cells. We conclude YC-1 decreases placental production of sFlt-1 and sENG and decreases endothelial dysfunction. It is a novel therapeutic candidate for preeclampsia.

1. Introduction

Preeclampsia is a leading cause of maternal and perinatal mortality and morbidity (Redman and Sargent, 2005; Sibai et al., 2005). A crucial step in the pathogenesis is the release of anti angiogenic factors sFlt-1 (Maynard et al., 2003) and soluble endoglin (Venkatesha et al., 2006) into the maternal vasculature. This leads to widespread endothelial dysfunction and multisystem organ injury and failure. Currently there is no medical treatment and delivery at preterm gestations is often required to stop disease progression (Chaiworapongsa et al., 2014a). A medical therapeutic that stabilizes the disease process and allows pregnancies to safely continue, could significantly improve fetal and maternal outcomes (Chaiworapongsa et al., 2014b).

The etiology of preeclampsia is believed to stem from abnormal placental trophoblast invasion into maternal uterine spiral arteriols (Redman and Sargent, 2005; Sibai et al., 2003). This is thought to result in inadequate placental perfusion and hypoxia and leads to increased secretion of sFlt-1 and soluble endoglin (sENG) (Redman and Sargent, 2005; Karumanchi and Bdolah, 2004). This premise is supported by the clinical observation that patients with preeclampsia have a 50–70% reduction in uteroplacental blood flow (Lunell et al., 1984). Furthermore, restricting uterine blood flow in pregnant rats increases serum sFlt-1 and induces a preeclamptic-like phenotype (Granger et al., 2006). Hypoxic-inducible factor-1α (HIF1α) is the master regulator of the hypoxic response and has been found to be increased in preeclampsia (Caniggia and Winter, 2002; Rajakumar et al., 2004). Over-expression of HIF1α in pregnant mice recapitulates the preeclamptic phenotype including elevated circulating sFlt-1, hypertension and proteinuria (Tal et al., 2010). Therefore, a medication that blocks HIF1α could represent a therapeutic strategy for preeclampsia, as it may decrease placental production of sFlt-1 and sENG (Rana et al., 2014).

Endothelial dysfunction is responsible for the maternal preeclampsia phenotype and results in widespread end organ injury (Borzychowski et al., 2006). It is likely caused by an increase in circulating antiangiogenic factors sFlt-1 and sENG and an elevation in the potent vasoconstrictor endothelin 1 (Nishikawa et al., 2000) and pro-inflammatory cytokine TNFα (Sanchez-Aranguren et al.,

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2014). Thus, a drug that can reduce endothelial dysfunction may also be useful as a treatment for preeclampsia.

YC1 3-(5′-hydroxymethyl-2′-furyl)-1-benzylindazole is a cardiovascular medication (Stasch et al., 2011) that quenches endothelial dysfunction (Galle et al., 1999) by inducing nitric oxide (Wohlfart et al., 1999), a potent vasodilator, and also independently activates its receptor guanylyl cyclase (Ramos-Espiritu et al., 2011). It has also been found to inhibit HIF1α (Yeo et al., 2003). Given its known vasoprotective properties and its ability to block HIF1α we hypothesize that YC-1 may reduce sFlt-1 and sENG secretion from primary human pregnancy tissues and rescue the endothelial dysfunction characteristic of preeclampsia. We therefore undertook functional studies in primary human tissues to examine the potential of YC-1 as a novel treatment for preeclampsia.

2. Materials and methods

2.1. Placenta explant culture

Human placental tissue was collected from three women with normal pregnancy at term and also three women with severe early onset preeclampsia (delivered at ≤34 weeks gestation). Preeclampsia was defined using the 2013 American College of Obstetricians and Gynecologists (ACOG) guidelines: the presence of hypertension >140/90 on two occasions 4 h apart and any of the following: proteinuria >300 mg/day, renal insufficiency, impaired liver function, thrombocytopenia or visual disturbance (ACOG, 2013). Written informed consent was obtained from all women and ethical approval was obtained from the Mercy Health Human Research Ethics Committee.

Villous explants were prepared as previously described (Brownfoot et al., 2014) and cultured in DMEM high glutamax (Life Technologies, Victoria, Australia) containing 1% antibiotic-antimycotic (Life Technologies) and 10% fetal calf serum (FCS) (Sigma, St Louis, United States). After 24 h placental explants were treated with 0, 1, 10, 100 μmol/L (μM) YC-1 (Sigma) for 72 h under 1% or 8% O_2 and 5% CO_2 at 37 °C. At the cessation of the experiment, excess culture media was removed by blotting before placental explant wet weight was determined. sFlt-1 and sENG levels were expressed as pg/mg placental tissue. Placental explant tissue was collected for RNA extraction.

2.2. Isolation and treatment of primary human umbilical vein endothelial cells (HUVECs)

Umbilical cords were collected from normal term placentas. The cord vein was infused with 10 ml (1 mg/ml) of collagenase (Worthington, Lakewood, New Jersey) and cells isolated as previously described (Brownfoot et al., 2014). Cells were cultured in M199 media (Life Technologies) containing 10% FCS (Sigma), 1% antibiotic-antimycotic (Life Technologies) and 1% endothelial cell growth factor (ECGS) (Sigma) and 1% heparin (Sigma) and used between passages 2–4. Cells were plated at 24,000/cm^2 and treated at 80% confluency with 0, 1, 10, 100 μM YC-1 for 24 h. A cell viability assay, MTS assay (lLife Technology) was performed and conditioned media was collected for assessment of sFlt-1 and sENG secretion and cell lysates collected for RNA extraction.

2.3. Isolation and treatment of primary human trophoblast cells

Term placentas were collected from women having elective caesarean sections. Human trophoblasts were isolated as previously described (Brownfoot et al., 2014; Tu'uhevaha et al., 2014). Primary trophoblasts were cultured in DMEM high Glutamax (Life Technologies) containing 10% FCS and 1% antibiotic-antimycotic (Life Technologies) on fibronectin (10 μg/mL; BD Biosciences, New South Wales, Victoria) coated plates. Cells were plated at 250,000/cm^2 and attached over 24 h before being washed in PBS (Brownfoot et al., 2014). Cells were treated with 0, 1, 10, 100 μM YC-1 for 24 h under 8% O_2 and 5% CO_2 at 37 °C. A cell viability assay, MTS assay (Life Technology) was performed and conditioned media was collected to assess sFlt-1 secretion.

2.4. Endothelial dysfunction

Endothelial dysfunction was induced by treatment of primary HUVECs for 2 h with 10 ng/ml TNFα (Sigma) followed by concurrent treatment with YC-1 at 0, 1, 10 or 100 μM for 24 h. For the leucocyte adhesion assay, THP-1 cells were pre-incubated with calcein (Merk Millipore, Darmstadt, Germany) for 30 min at 37 °C. THP-1 cells were then washed and collected before being added to primary HUVECs treated with 10 ng/ml TNFα with and without YC-1 0, 1, 10 or 100 μM at 1×10^6 per ml and cultured for 45 min. A Fluostar omega fluorescent plate reader (BMG labtech, Victoria, Australia) was used to detect fluorescence (quantify adhesion) and an EVOS FL microscope (Life Technologies) was used to capture images.

2.5. ELISA analysis

Concentrations of sFlt-1 and sENG were measured in conditioned cell/tissue culture media using the DuoSet VEGF R1/Flt-1 kit (R&D systems by Bioscience, Waterloo, Australia) and a DuoSet Human Endoglin CD/105 ELISA kit (R&D systems) as per manufacturer's instructions.

2.6. RT-PCR

RNA was extracted from trophoblasts, placental explants and HUVECs using an RNeasy mini kit (Qiagen, Valencia, CA) and quantified using the Nanodrop ND 1000 spectrophotometer (NanoDrop technologies Inc, Wilmington, DE). 0.2 ug of RNA was converted to cDNA using Superscript VILO cDNA synthesis kit (Life Technologies) as per manufacturer guidelines.

Taqman gene expression assays for MMP 14 and HIF1α were used (Life Technologies). RT-PCR was performed on the CFX 384 (Bio-Rad, Hercules, CA) using FAM-labeled Taqman universal PCR mastermix (Life Technologies) with the following run conditions: 50 °C for 2 min; 95 °C for 10 min, 95 °C for 15 s, 60 °C for 1 min (40 cycles). A Sybr gene expression assay for sFlt-1 e15a was used. Primers were designed as previously described (Geneworks, South Australia, Australia) (Whitehead et al., 2011). RT-PCR was performed using the following run conditions: 95 °C for 20 min; 95 °C for 0.01 min,60 °C for 20 min, 95 °C for 1 min (39 cycles), melt curve 65 °C–95 °C at 0.05 °C increments at 0.05 s.

All data were normalized to GAPDH as an internal control and calibrated against the average C_t of the control samples. Results expressed as fold change from control.

2.7. Western blot

HUVECs were cultured in a T75 flask until they were 70% confluent. They were treated with 100 μM YC-1 or vehicle control for 24 h. The cells were washed with ice-cold PBS and removed from the flask using a cell scraper. Cell pellets were centrifuged for 5 min at 450 G before supernatant was discarded and lysis buffer added for 15 min. Cell lysate was centrifuged for 5 min at 450 G and supernatant discarded before fresh lysis buffer was added and lysate drawn through a 25 gauge hypodermic needle 5 times. Lysate was then centrifuged for 20 min at 10,000 G. The supernatant containing the cytoplasmic fraction was removed and extraction