Effect of placental growth factor on trophoblast integration into endothelial cell networks in the presence of inflammation

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Background

- Preeclampsia is a leading cause of maternal and perinatal morbidity and mortality, affecting 3 - 5% of pregnancies.
- The pathogenesis of preeclampsia is a ‘two stage’ process

Stage 1: Abnormal placental development with incomplete remodelling of uterine spiral arterioles by trophoblast cells
Stage 2: The abnormal placenta then produces factors which contribute to the development of the maternal syndrome
Background

Placental growth factor (PLGF) is a pro-angiogenic factor. PIGF rises to a peak at approximately 30 weeks’ gestation and then decreases. In women who develop preeclampsia, PIGF is lower in early pregnancy.
• PlGF has been used as a treatment in experimental models of preeclampsia with promising results
• As circulating PlGF is low in advance of clinical symptoms, replacement of PlGF is hypothesised as a preventative treatment. However, the role of PlGF in early placental development remains unclear.
Effect of PlGF on trophoblast and endothelial cell networks

- Single culture of HTR8/SV neo cells on matrigel showed trophoblast network formation was improved 2-fold in 2% oxygen with high dose (100 ng/mL) rhPlGF (p = 0.02)
- Integration between trophoblast and endothelial cells was unaffected by rhPlGF in both 2% oxygen and 21% oxygen conditions compared to control
- Integration was also no different when comparing 2% to 21% oxygen conditions

Figure: HTR8/SVneo on matrigel. Length of trophoblast network structures measured using Angiogenesis Analyzer on Image J. i) Untreated ii) PlGF 10 ng/mL iii) PlGF 100 ng/mL
Aim

• To investigate the effect of supplemental placental growth factor in an *in vitro* model of early maternal blood vessel remodelling in the presence of TNF-α

• TNF-α is elevated in women with preeclampsia and induces an experimental model of preeclampsia in animals
Methods

• Tissue culture plates were coated with undiluted Matrigel
• Fluorescent labelled (RED) uterine myometrial microvascular endothelial cells (1 X 10^5/well) were seeded into each well.
• Capillary networks formed, then fluorescent (GREEN) labelled trophoblast HTR8/SVneo cells (1 X 10^5/well) were added.
• Wells were exposed to TNF-α and treated with recombinant human PlGF at concentration of 10 ng/mL
Methods

Uterine myometrial microvascular endothelial cell networks

HTR8/SVneo trophoblast integrate into endothelial cell network

% integration = Trophoblast area (µm²)/Endothelial area (µm²)
Cell integration

* Control vs TNF p = 0.01
** Control vs TNF + PlGF p = 0.03
Angiogenic factors

- Addition of TNF-α decreases PlGF, increases VEGF and reduces sFLT-1 expression in conditioned media measured by ELISA
- PlGF did not reverse the effect of TNF-α upon VEGF or sFLT-1 protein expression
- PlGF alone does not VEGF or sFLT-1 protein expression

*control vs TNF p = 0.02; ** control vs TNF + PlGF p = 0.02; *** control vs TNF p = 0.01
mRNA expression of cell surface molecules

• Expression of integrin α1β1 and toll-like receptor 3 was unchanged across treatment wells

• Vascular cell adhesion molecule-1 (VCAM-1) expression was increased by TNF-α exposure but this was not changed by the presence of PlGF

• PlGF alone does not affect VCAM-1 expression

*Control vs TNF p = 0.0004
** Control vs TNF + PlGF p = 0.0003
Conclusion

- Supplemental PlGF does not abrogate the inhibitory effects of TNF-α on first trimester trophoblast and uterine myometrial microvascular endothelial cell interactions.
- Clinical implication – Replacement of PlGF early in pregnancy may not improve placentation.
Acknowledgements

• NHMRC postgraduate scholarship
• PEARLS Preeclampsia Research Laboratories