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Resolvin D2 Protects Placental Cells from Inflammation by Modifying the Mitochondria in Hypertensive Disorders of Pregnancy

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Background: Hypertensive Disorders of Pregnancy (HDP) contributes to 70,000 maternal and 500,000 fetal deaths annually worldwide. HDP is characterized by poor placental trophoblast invasion and migration followed by a maternal inflammatory response. Increased pro-inflammatory cytokine tumor necrosis factor alpha (TNFα) can promote mitochondrial dysfunction. Nuclear Factor Erythroid-2-Realted-Factor-2 (NRF2) regulates anti-inflammatory gene expression by making Mitochondrial Transcription Factor A (TFAM), a controller of mitochondrial replication. Dysregulation of the NRF2-TFAM pathway may drive mitochondrial dysfunction. Resolvin D2 (RvD2), an omega-3 (n-3) fatty acid-derived specialized pro-resolving mediator (SPM), is known to promote anti-inflammatory and pro-repair processes; however, the mechanism between SPMs regulating inflammation and mitochondrial function during HDP is unclear.

Significance of the Problem: Little is known about the effects of TNFa and RvD2 on trophoblast cells. This study fills the gap in knowledge by demonstrating that RvD2 improves placental cell migration without creating cytotoxicity. RvD2 protects the mitochondria by regulating anti-inflammatory genes, replicating mitochondria, and mitigating oxygen consumption rates. SPMs represent potential therapeutic targets for modulating inflammation and mitochondrial dysfunction.

Hypothesis: We hypothesize that RvD2 mitigates TNFα-driven inflammatory response and mitochondrial dysfunction by modulation of NRF2-TFAM pathway in trophoblasts.

Experimental Design: Human-derived first and third trimester trophoblast cells were subjected to TNFα (10-100 ng/mL) in vitro. RvD2 (10-100nM) was applied as a treatment strategy to mitigate inflammation and mitochondrial dysfunction. Migration assays were performed to measure trophoblast function. qPCR and immunoblots were used to measure NRF2 and TFAM mRNA and protein expression. Seahorse® was used to measure oxygen consumption rates (OCAR). LDH and CCK8 colorimetric assays were used to measure cytotoxicity and cell viability. One-way ANOVA or student t-test was performed.

Results: RvD2 significantly improved migration in trophoblast cells while TNFα decreased it (RvD2 42% vs. TNFa: 32%; p=0.019). Trophoblasts subjected to TNFα demonstrated an increase in TFAM mRNA and protein expression levels; treatments with RvD2 mitigated it (TFAM relative expression to β-Actin: TNFα: 0.002 vs. TNFa+RvD2: 0.001; p=0.026). Nuclear NRF2 protein expression increases treatments of TNFα but decreases with treatments of RvD2. OCAR for maximal and basal respiratory rates and ATP production is modulated by RvD2 treatments (ATP OCAR: RvD2: 119.7 vs. TNFα: 295 vs. RvD2+TNFa: 173; p=0.010; pmol/min/mg/mL). TNFa or RvD2 did not significantly alter cellular cytotoxicity or viability.

Conclusion: RvD2 exhibits a protective role through modulation of the NRF2-TFAM pathway in the mitochondria of trophoblasts during TNFα-driven inflammatory insults and can functionally alter trophoblast migration.