DIABETES/PHYSIOLOGY/PREMATURITY

Abstracts 44 – 52

Moderators: Roger Newman, MD; Arthur Evans, MD

44 PKCβ2 inhibition reduces neural tube malformations and suppresses caspase activation

Yuanning Cao[1], Zhiyong Zhao[1], Richard L. Eckert[1], E. Albert Reece[1]
[1]University of Maryland School of Medicine, Baltimore, MD

OBJECTIVE: Neural tube defects (NTDs) in infants of diabetic mothers are associated with increased programmed cell death (apoptosis) in the neuroepithelium during early embryogenesis leading to diabetic embryopathy. Recent data suggests that protein kinase Cβ2 (PKCβ2) plays a role in diabetic embryopathy. We propose that it may regulate apoptosis. Apoptosis in diabetic embryopathy is associated with increased caspase-8 and -3 activity and increased tBid cleavage.

STUDY DESIGN: To test this hypothesis, mouse embryos at embryonic day 7.5 (E7.5) were cultured under hyperglycemic conditions (400 mg glucose/dl) in the presence or absence of the PKCβ2 inhibitor (PKCβ-I, 50 nM) for 48 h.

RESULTS: The neural tube malformation rate was 58.3% in embryos maintained under hyperglycemic conditions. The high rate of neural tube malformation was associated with increased apoptosis including activation of caspase-8 and -3, and increased Bid cleavage to form tBid. Treatment with PKC inhibitor reduced the malformation rate to 26.7% which is similar to the rate observed in embryos maintained under euglycemic conditions (150 mg glucose/dl), and also reduced caspase activation and Bid cleavage to control levels.

CONCLUSIONS: These findings indicate that PKCβ2 is required for hyperglycemia-induced neural tube malformation via a mechanism that involves regulation of caspase-8 associated apoptosis.

45 Role of HIF-1alpha in maternal hyperglycemia-induced embryonic vasculopathy

Peixin Yang[1], E. Albert Reece[1]
[1]University of Maryland School of Medicine, Baltimore, MD

OBJECTIVE: Maternal diabetes adversely impacts embryonic vasculogenesis resulting in embryonic vasculopathy. This, in turn, leads to malformations and/or embryonic lethality. HIF-1α, an oxygen sensitive subunit of HIF-1, is essential for normal embryonic vasculogenesis. The purpose of our study is to determine whether HIF-1α plays a role in diabetic embryonic vasculopathy.

STUDY DESIGN: Protein and mRNA levels of HIF-1⟨ were determined in embryonic day 7 (E7) and E8 conceptuses from non-diabetic and diabetic mice. E7 conceptuses were cultured for 24h or 48h under euglycemic (150 mg/dl glucose) and hyperglycemic (300 mg/dl) conditions in the presence or absence of 0.5 ⌈l or 1 ⌈l (1x10^7 IFU/ml) AdCA5 per 1ml culture medium, or in the presence or absence of 2.0 ⌈g/ml human recombinant thioredoxin (Trx), an endogenous anti-oxidant protein. AdCA5 is an adenovirus encoding a constitutively active form of HIF-1⟨.

RESULTS: Maternal diabetes significantly reduced HIF-1⟨ protein in both E7 and E8 conceptuses. In contrast, maternal diabetes did not alter HIF-1⟨ mRNA levels, suggesting that diabetes regulates HIF-1⟨protein stability. The administration of 0.5 ⌈l AdCA5 increased hyperglycemia-reduced vasculature morphological scores. In addition, 1 ⌈l AdCA5 completely reversed hyperglycemia-reduced vasculature morphological scores, and AdCA5 reversed hyperglycemia-reduced VEGF protein expression. Trx treatment reversed hyperglycemia-reduced HIF-1⟨ levels.

CONCLUSIONS: We conclude that reduced HIF-1⟨ plays a critical role in the induction of diabetic embryonic vasculopathy and hyperglycemia-induced VEGF reduction. Because Trx blocks hyperglycemia-reduced HIF-1⟨, oxidative stress is implicated in the reduction of HIF-1⟨ by hyperglycemia.

46 Progesterone receptor membrane component 1 (PGRMC1) inhibits Ca2+ mediated cell death in human cytotrophoblast cells

Amy Murtha[1], Chad Grotegut[1], R. Phillips Heine[1], Liping Feng[1]
[1]Duke University Medical Center, Durham, NC

OBJECTIVE: Clinical use of progesterone has been the focus of intense clinical research in preterm birth prevention yet little is known about its mechanistic role. Our preliminary work demonstrates that progesterone treatment of chorion cells provides protection from Ca^{2+} mediated cell death and that these cells express high levels of the novel non-nuclear progesterone receptor membrane component 1 (PGRMC1). This effect occurs consistently in cytotrophoblast cells (HTR-8/SVneo) which contain PGRMC1 but lack the classic nuclear progesterone receptors (PR), A and B. Our objective was to determine the role of PGRMC1 in calcium-induced apoptotic cell death by depleting PGRMC1 through small interfering(si) RNA in cell culture.

STUDY DESIGN: A first trimester cytotrophoblast cell line, HTR-8/SV neo, a gift from Dr C. H. Graham (Queen's University, ON), was transfectedwith either scrambled control siRNA or siRNA directed against PGRMC1. The relative amount of PGRMC1 after PGRMC1 siRNA treatment was determined by Western blot. HTR-8/SV neo cells were pre-treated with or without progesterone (R5020) (10^{-6} or 10^{-7} M) for 1 hour (h) in phenol-red free media followed by calcimycin (5μM) treatment for 24 h following PGRMC1 siRNA or control siRNA treatment. Apoptosis was evaluated by Western blot for cleaved caspase 3. All experiments were performed in triplicate.

RESULTS: PGRMC1 siRNA significantly reduced PGRMC1 protein levels ($P < 0.05$)compared to control siRNA. Treatment with PGRMC1 siRNA significantly attenuated R5020's ability to suppress apoptosis induced by calcimycin (5uM) in HTR8/SV neo cells (figure 1).

CONCLUSIONS: These findings suggest an important role of PGRMC1 in cell survival mediated through calcium homeostasis. This work indicates a key regulatory function for this non-nuclear PR in pregnancy tissue and extends current knowledge of the molecular mechanisms by which progesterone may act in preterm birth prevention.

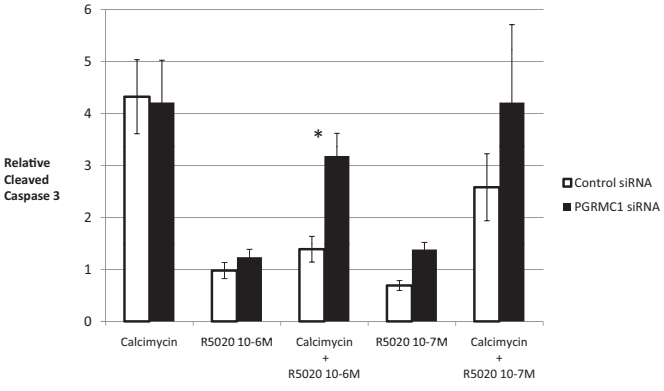

Figure 1. Pretreatment with PGRMC1 siRNA results in diminished protective effect of progesterone (R5020) from calcium mediated cell death (* P=.02).