Theca Externa

Payal Shah, MS, Kadden Heinrich Kothmann, BS, Cassandra Suzanne Skenandore, MS, Luke Browning, BS, Camille Goblet, MS, Laura Chau, BS, Matthew Grizzaffi, BS, Anne Elizabeth Newell-Fugate, DVM, PhD.
Dept. Veterinary Physiology and Pharmacology, TEXAS A&M UNIVERSITY, College Station, TX, USA.

Western-style diets (WSD) cause cardiometabolic disease and hyperandrogenemia in reproductive-age women. A promising alternative for improved fertility in obese women may derive from the nutraceutical, coconut oil. We hypothesized that dietary coconut oil included in a high fat diet would mitigate hyperandrogenemia due to obesity through depression of androgen steroidogenesis in theca externa and adrenal cortex. Seventeen sexually mature female Ossabaw pigs were divided into 3 diet groups: control (n=6; C; 2200 kcal/pig/day), WSD (n=5; WSD; 5000 kcal/pig/day), or high fat coconut oil diet (n=6; COC; 5000 kcal/pig/day). Ingredients in WSD and COC diets were the same except 9% of calories in WSD were from lard and 9% of calories in COC were from coconut oil. Pigs were fed for 9 cycles (~ 8 mos) and fasting blood was collected at cycle 0 (baseline), 7, and 9. After cycle 7, ovarian steroidogenesis was suppressed by oral progestogen for 18 days. On day 18 while ovarian steroidogenesis was still suppressed, an ACTH stimulation was performed. On day 19, progestogen was withdrawn and 7.5 μg/kg IM dexamethasone was given every 12 hours to suppress adrenal steroidogenesis. On day 20, 3000 IU/m^2 human chorionic gonadotropin (hCG) was given IV. Pigs underwent a washout cycle post-stimulations and were euthanized when they had dominant ovarian follicles in cycle 9 for collection of follicular fluid (FF), theca externa, and adrenal cortex. FF was assessed for steroid hormones by LC-MS/MS. Adrenal cortex and theca cells were cultured as follows for 48 hours after which cells and media were collected: 1) theca: control, insulin (I; 100 ng/ml), LH (10 ng/ml), or LH+I (10 ng/ml + 100 ng/ml); 2) adrenal: control or 1 μM ACTH. WSD pigs had a protracted estrous cycle length and increased FF testosterone, dehydroepiandrosterone sulfate, androstenedione (A4), androstenediol, and allo-pregnanolone (Allo-Preg) compared to C pigs. Both serum and cell culture media A4 concentrations were higher in response to ACTH in WSD pigs. By 72 hours post-hCG, COC pigs had higher serum A4 than C or WSD pigs, but *in vitro* LH yielded decreased cell culture media A4 in both WSD and COC pigs. COC pigs had increased FF dihydrotestosterone and Allo-Preg compared to C pigs. These results suggest that dietary coconut oil administration in obese females may depress excessive androgen steroidogenesis by adrenal cortex but may only partially mitigate excessive androgen production by theca externa.

Reproductive Endocrinology

OVARY, TESTES, AND IMPACT OF HORMONES ON METABOLIC FUNCTION

Exploring NCX4040, an Aspirin Derivative, as a Potential Treatment for Benign Prostatic Hyperplasia

Preksha Vaibhav Shahagadkar, B. Tech Biotechnology[1], Gnanasekar Munirathinam, PhD[2].
[1]UNIVERSITY ILLINOIS COM-ROCKFORD, Rockford, IL, USA, [2]University of Illinois at Chicago, Rockford, IL, USA.

Benign Prostatic hyperplasia (BPH) is a leading cause of lower urinary tract symptoms which affects men above 50 years of age. Chronic inflammation and abnormal proliferation of stromal and epithelial cells are implicated in BPH disease onset. The symptoms of BPH include back pain and difficulty in emptying bladder. Finasteride, sildenafil and tamsulosin are some of the drugs used to ease difficulty urinating and relax the muscles of the gland. Transurethral resection of the prostate or laser surgery can be performed to treat severe symptoms. However, these therapies have deleterious effects such as low blood pressure, ejaculatory dysfunction, and lump formation. Hence, there is an unmet need for potential drugs against BPH. Nonsteroidal anti-inflammatory drugs (NSAIDs) have proven to be effective in cancers but their applicability in BPH condition is yet to be fully explored. Aspirin, one of the NDSAIDs, has anti-tumor and anti-inflammatory properties at higher doses. NCX4040, a nitric oxide releasing derivative of aspirin, could prove to be effective against BPH, since it can inhibit abnormal cell proliferation and serve as a vasodilator. We hypothesize that NCX4040 would be an effective drug to treat BPH. BPH-1 epithelial and WPMY-1 stromal cells were used as *in vitro* models of BPH. MTT assay was performed to check the inhibitory effect of NCX4040 and blocking agents like catalase and N-acetyl-L-cysteine (NAC) were explored on cells after treatment. Clonogenic assay was done to explore the colony formation ability of cells. Spheroid assay was performed to analyze the anti-proliferative effect of NCX4040. Annexin V/PI and cell cycle analysis was performed to check for apoptosis and cell cycle arrest in the cells. Western blot was done to assess the signaling molecules altered by NCX4040 in BPH-1 cells. Confocal immunofluorescence was employed to analyze the dynamics of actin filaments after treatment in cells. Our studies revealed that NCX4040 inhibited the cell viability of BPH-1 and WPMY-1 in a dose dependent manner with IC50 predicted at 5μM and 2.5μM respectively. Of note, catalase and NAC blocked the effect of NCX4040 on prostate cells. Colony formation assay result implied a gradual decrease in the number of colonies of cells treated with NCX4040 with 2.5μM and 5μM doses. Spheroid assay in BPH-1 cells showed inhibitory effects after treatment. Cell cycle analysis by flowcytometry inferred that cell cycle arrest at G2/M phase and annexin V analysis indicated that activation of apoptosis in cells following treatment. Phalloidin staining showed decrease in the actin filament intensity in cells. At the molecular level, NCX4040 downregulated the expression of key markers such as RhoA, p65, COX-2, PCNA, Cyclin D3, and PDE-5 in BPH-1 cells. Taken together, NCX4040 could be used as a potential agent to manage BPH with minimal side effects, which needs further evaluation in animal models.

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Fadrozole-Mediated Sex Reversal Induces PAX2$^+$Undifferentiated Supporting Cells in Female