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MON-039

Polycystic ovary syndrome (PCOS) is the most common cause of anovulatory infertility, classically presenting with disrupted ovulation, polycystic ovaries, and androgen excess, as well as many non-reproductive comorbidities. For instance, PCOS patients exhibit increased stress reactivity and higher rates of depression and anxiety compared to the general population. The prenatal anti-Mullerian hormone (pAMH)-induced model of PCOS was recently shown to recapitulate reproductive phenotypes in female mice, however little remains known about the consequences of pAMH exposure. We first aimed to expand upon this model by investigating pAMH-induced effects on offspring of both sexes. Pregnant dams on a C57Bl/6 background received daily i.p. injections of either AMH (0.12 mg/kg/d) or VEH late in gestation. Offspring were born into 4 groups (pAMH vs. VEH females, pAMH vs. VEH males) and assessed starting at weaning for changes in body weight, anogenital distance, pubertal onset, estrous cyclicity, fertility, and reproductive senescence. Statistical differences were determined by t-test or 2-way ANOVA when applicable, and significance set at $p<0.05$. As expected, pAMH increased anogenital distance in females but not males. Pubertal onset was delayed not only in females as previously reported, but also in males. Additionally, pAMH adult females showed significant disruptions in estrous cycling at P60 (increased time spent in diestrus, decreased number of cycles, increased cycle length), only mild disruptions by P90, then robust disruptions at 8 mo, 10 mo, and 12 mo of age that were distinct from reproductive senescence. When paired with wildtype untreated mates for a fertility assay starting at 3 mo of age, pAMH females had smaller and fewer number of litters, while pAMH males showed only delayed plugging behavior. Although pAMH males showed no difference in testis weight, pAMH females also had significantly reduced ovarian and uterine weights in diestrus. Interestingly, during the fertility assay, we found increased fetal death from both the pAMH females and males, even though pAMH males were paired with wildtype untreated females. We hypothesized that the increased fetal death could be the result of an pAMH-induced stress phenotype in both sexes. Using a simple stress response test measuring defecation and urination during exposure to a novel environment, we found that pAMH robustly increased stress response in both sexes at multiple timepoints. We also assessed glucocorticoid response to a restraint stress paradigm in adult females. While we observed no differences in baseline serum corticosterone levels, the pAMH group showed increased peak levels followed by a prolonged elevation levels after 2 hr. Together, these results enhance existing knowledge of the effects of pAMH exposure by demonstrating alterations in both male and female mice on both reproductive and non-reproductive measures.

Tumor Biology

TUMOR BIOLOGY: GENERAL, TUMORIGENESIS, PROGRESSION, AND METASTASIS

Optimization of Experimental Conditions for Mimicking Hypoxia in Cultured Breast Cancer Cells by Using Cobalt(II) Chloride ($CoCl_2$)

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SAT-124

Hypoxia is a common phenomenon in solid tumor development caused by a decrease in either oxygen concentration or oxygen pressure as a result of rapid tumor cell growth. Hypoxia is characterized by stabilization of the alpha subunit of the hypoxia-inducible factor (HIF-1α) and its nuclear translocation and heterodimerization with HIF-1β. Activation of this signaling pathway involves multiple downstream effectors including carbonic anhydrase 9 (CA9, *s.* CAIX). A reliable method to mimic hypoxia utilizes cobalt(II) chloride ($CoCl_2$), which directly induces the expression of HIF-1α. The aim of this study was to optimize the experimental conditions for $CoCl_2$ treatment of breast cancer cells *in vitro* using three human breast cancer cell lines (MDA-MB-231, T-47D, and MCF-7 cells). We performed time- and concentration-response experiments, using various concentrations of $CoCl_2$ (50, 100, 200, and 300 μM) for 24 and 48 hours, and measured the expression of HIF-1α and CA9 by qRT-PCR and Western blot analyses. Results demonstrated that $CoCl_2$ downregulated HIF-1α mRNA levels but upregulated CA9 mRNA levels in a concentration- and time-dependent manner. Concomitantly, $CoCl_2$ treatment resulted in a significant induction of HIF-1α protein levels. We further investigated the effect of the $CoCl_2$ concentrations listed above on cell apoptosis using an *in situ* apoptosis detection kit. The results demonstrated that concentrations of $CoCl_2$ up to 100 μM had no significant effect on cell apoptosis.

Neuroendocrinology and Pituitary

PITUITARY TUMORS: TRIALS AND STUDIES

Intrapatient ACTH Variability in Cushing's Disease: Prognostic Significance

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OR23-01

Introduction: In patients with Cushing's Disease (CD), intrapatient variability of hormone measurements creates significant clinical challenges, therefore multiple measurements are recommended.[1] Urinary and salivary cortisol variation has been well described. However, intrapatient variation of adrenocorticotropic hormone (ACTH) in CD remains unknown. In CD patients, ACTH levels are inherently elevated from baseline but the coefficient of diurnal variation is reduced.[2]Additionally, at each diurnal time point, these exists a significant variation around the mean for the ACTH levels. In this study, we first analyzed the intrapatient variablility of ACTH at each diurnal timepoint in patients with CD. CD is primarily a disorder of ACTH excess, and treatment directed at pituitary adenomas would presumably perturb ACTH levels prior to affecting serum or urine cortisol. We hypothesized that the coefficient of variation at each diurnal time-point can help predict remission from CD following surgery.

Methods: We conducted a retrospective review of patients (n = 645) who had histopathologically confirmed diagnosis of CD from 2005-2019 (NCT NCT00060541). We selected patients that had ≥ 3 plasma ACTH values over a 7 day span prior to surgical or medical intervention. We grouped the ACTH measurements into morning (AM) and midnight (PM) values to account for diurnal variation in ACTH secretion. We then analyzed post-operative hormone measurements performed every 6 hours prior to administration of replacement corticosteroids. Remission was assigned to patients with nadir serum cortisol level ≤5 mcg/dL within ten days post-operatively[3,4].
Results: We found 54 patients with multiple PM (n = 27) and AM (n = 41) ACTH measurements within a 7 day span. We found that the median coefficient of variation (CV) of intra-patient variability was 19.7% (N=41) (95% CI:12.5-27.5) for the AM and was 24% (N=27) (95% CI: 9.6-31.8) for the PM. Age, the number of tests, or the length of test period were not correlated with CV or absolute levels of ACTH. The intraclass correlation coefficient (ICC) of the AM data set was 0.59 and the PM data set was 0.79 which demonstrates a good and excellent reliability respectively. We found that that, in general, 30-60% decrease from pre-operative ACTH levels predicted remission from CD. ACTH decrease >50% on POD2 and 3 had 100% specificity and sensitivity in predicting remission. The decrease in ACTH preceded cortisol nadir in 3/10 patients by 24 hours.
Conclusion: We found significant intra-patient variability in plasma levels of ACTH at individual diurnal timepoints in CD patients. We also found that the change in ACTH >50% on POD2 or 3 is an excellent predictor of remission from CD.

Reproductive Endocrinology

OVARIAN FUNCTION — FROM OLIGOMENORRHEA TO AMENORRHEA

Emergence of Ovarian Hyperandrogenism and Luteal Insufficiency Following ESR1 Knockdown in the Mediobasal Hypothalamus of Adult Female Rhesus Monkeys

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OR31-05

Diminished estradiol (E2) negative feedback action by neuronal ESR1 in the arcuate nucleus (ARC) of the mediobasal hypothalamus (MBH) is hypothesized to cause gonadotropin-releasing hormone (GnRH) hypersecretion, and thus LH excess, contributing to ovarian hyperandrogenism in polycystic ovary syndrome (PCOS). In primates, including humans, however, the mediating estrogen receptor is unknown. Thus, to test the hypothesis that diminished E2 action on ARC ESR1 contributes to female primate ovarian hyperandrogenism, eleven, ovary intact, adult female rhesus macaques, pair housed with female peers, received five 12µl MRI-guided MBH infusions into the rostral-to-caudal extent of both right and left ARC. Each infusion comprised gadolinium contrast agent and ~3-4 x 10^{10} adeno-associated virus 8 (AAV8) particles containing either a shRNA specific for ESR1 (n=6, ERaKD) or scrambled shRNA (n=5, control). Mid-surgery MRI scans identified targeting accuracy. 2-2.5 years following AAV8 infusion, EIA-determined P4 values were obtained from twice weekly serum samples; samples obtained during the follicular phase of menstrual cycles or anovulatory periods were submitted to liquid chromatography, tandem mass spectrometry (LCMS) for additional steroid hormones. LCMS-determined values were also obtained 0 hours (h) and 24 h following an IM injection of 200IU hCG. Both ERaKD (28.5 ± 1.3 days, mean ± SEM) and control (34.0 ± 3.3 days) female groups exhibited comparably regular menstrual cycles. ERaKD exhibited higher circulating levels of LH (2.8 ± 0.2 ng/ml, p=0.03), androstenedione (A4, 0.43 ± 0.03 ng/ml, p=0.03) and testosterone (T, 0.23 ± 0.03 ng/ml, p=0.09), and LH/FSH ratio (1.7 ± 0.2, p=0.05) compared to controls (LH, 2.1 ± 0.4; A4, 0.30 ± 0.05; T, 0.18 ± 0.01 ng/ml; LH/FSH 1.3 ± 0.2). Following an ovarian androgen-stimulating hCG injection, ERaKD 24-h peak levels for T (0.28 ± 0.01 ng/ml) were higher (p=0.03) compared to controls (0.21 ± 0.01 ng/ml). In addition, luteal insufficiency emerged in ERaKD females, with mean (2.4 ± 0.3 ng/ml), peak (3.6 ± 0.4 ng/ml) and area-under-the-curve (AUC, 23.2 ± 4.2 ng/ml/days) P4 values diminished compared to controls (mean, 3.6 ± 0.1, p=0.01; peak 5.7 ± 0.1 ng/ml, p=0.01; AUC, 43.7 ± 6.7 ng/ml/days, p=0.03). Taken together, these results suggest that knockdown of ARC ESR1 disrupts Gn stimulation of ovarian function, contributing to female monkey ovarian hyperandrogenism and menstrual cycle impairment emulating PCOS in women.

Bone and Mineral Metabolism

NEW INSIGHTS INTO PTH AND CALCIUM RECEPTOR SIGNALING

The Roles of GNAQ and GNA11 in Calcium-Sensing Receptor (CaSR) Signalling

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OR07-06

The G-protein subunits $G\alpha_{11}$ and $G\alpha_{q}$, which share >90% peptide sequence identity and are encoded by the *GNA11* and *GNAQ* genes, respectively, mediate signalling by the calcium-sensing receptor (CaSR), a class C G-protein coupled receptor (GPCR) that regulates extracellular