Bone and Mineral Metabolism

BONE DISEASE FROM BENCH TO BEDSIDE

Wnt Inhibition Decreases Trabecular Bone in a Mouse Model of Fibrous Dysplasia

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Background: G protein-coupled receptor (GPCR) signaling mediates a wide spectrum of physiological functions, including bone development and remodeling. Fibrous dysplasia (FD) is a common skeletal dysplasia where normal bone and bone marrow are replaced by fibrous tissue and expansile trabecular bone lesions. The craniofacial bones are often involved, leading to pain and facial deformities. FD is a mosaic disease caused by a somatic mutation in the *GNAS* gene encoding the G-protein alpha subunit (Gsα) that leads to constitutive activation of the G_s signaling pathway. Unfortunately, FD has no effective medical treatments.

Major challenges have hampered the development of pharmacologic strategies that specifically target *GNAS* or the $G_s\alpha$ protein. We previously developed the Col1(2.3)/Rs1 mouse model (Rs1) in which the G_s signaling pathway is activated specifically in bone by an engineered GPCR protein. These mice showed increased trabecular bone formation with loss of marrow space and cortical bone, which strongly resembles human FD (1–4). There was also a dramatic increase in the number of immature osteoblasts present in the FD lesions, suggesting that activation of G_s signaling caused an accumulation of these cells. Our prior studies showed increased Wnt signaling, which may be a major driver of this effect. Furthermore, blocking the G_s signaling could reverse the bone phenotype, providing proof-of-concept for finding drugs that could reverse the phenotype. Therefore, we administered the Wnt inhibitor LGK974, currently used in human clinical trials, to the Rs1 mice to test if the FD lesions could be pharmacologically reversed.

Methods: We administered LGK974 in 4-week-old Rs1 and non-Rs1 mice. We used a low dose (5mg/kg) for 8 weeks or high dose (30mg/kg) for 4 weeks. The mice were evaluated by histology and micro computed tomography (micro-CT) for mineral density (mg/cm^3), bone volume (mm^3), and trabecular thickness (um).

Results: LGK974 decreased β-catenin levels in bone on western blots. In the low-dose group, the histology and micro-CT showed no statistically significant differences between drug and control groups. In the high-dose group, the micro-CT showed significantly decreased trabecular bone thickness (p=0.0364, n=3) in the drug-treated group (22±2μm) compared with controls (17±2μm). Furthermore, histology showed resorption of the abnormal bone; however, the fibrocellular infiltrate in the Rs1 mice was still present.

Conclusions: Wnt inhibition can lead to decreased fibrous dysplastic bone, but separates abnormal bone formation from the fibrocellular infiltrate. These results provide new insight into understanding interactions between the Wnt and G_s signaling pathways in FD pathogenesis and bone formation.

References: 1. Hsiao EC et al. *PNAS*. 2008. 2. Hsiao EC et al. *JBMR*. 2010. 3. Schepers, Hsiao EC et al. *Blood*. 2012. 4. Cain CJ et al. *Endocrinology*. 2016.

Neuroendocrinology and Pituitary

CASE REPORTS IN SECRETORY PITUITARY PATHOLOGIES, THEIR TREATMENTS AND OUTCOMES

The Ever Confusing Cushing's Work Up: Is It Real? Is It Pseudo Cushing's'? Or Could It Be Factitious

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The Ever Confusing Cushing's Work Up: Is it real? Is it pseudo Cushing's, or could it be factitious?

Abstract Keywords:
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Case Presentation:

A 50 year old female initially presented with progressive weight gain and mood swings. She had normal 24 h urine cortisol, but an elevated midnight serum cortisol. She underwent transphenoidal surgery for a presumed ACTH-dependent Cushing's disease. Pathology was not supportive of a pituitary adenoma, showing adenohypophyseal tissue with focal expansion of the acini. The surgery was complicated by hypothyroidism and growth hormone deficiency. She was able to weaned off of the steroids after a few months. She had recurrence of her initial symptoms, she was found to have elevated late evening and morning cortisol levels. She underwent a bilateral adrenelactomy for "recurrence of the cyclical Cushing's symptoms." She was started on HC replacement; 10 mg AM and 2.5 mg PM, florinef 0.05 mg daily. She slowly lowered the hydrocortisone dose, and as a result lost 120 lbs.

Three years later she presented with fatigued and gaining weight, by that time she was on Hydrocortisone 3.75 mg AM, 1.25 mg evening, and fludrocortisone 0.1 mg/day. ACTH was 355 (6–48 pg/ml), serum cortisol 10 (8–19 ug/dl) on Hydrocortisone and < 1.0 ug/dl off cortisone. The 24 h urine free cortisol < 1.0 (10–24 ug/34h), and 17 OH-corticosteroids < 4.8(4–14 mg/dl). A possible adrenal remnant was seen on abdominal CT, surgically removed of the lesion showed a lipoma.

She was referred to Neurosurgery for a second pituitary surgery for the concern Cushing's recurrence. A pituitary MRI revealed a small potential microadenoma. The small dose of hydrocortisone was held for 48 h and an 8 AM test dose: Serum cortisol < 1.20 mcg/dl (3–18), ACTH 1,077 pg/ml (5–72), 24 h urine cortisol < 1.5 mcg/24h (3.5–45), 24 h urine cortisone 10 mcg/24h (17–129), and two midnight salivary cortisol were 128 and 265 ng/dl (< 100 ng/dl). There was a concern raised by the laboratory for a contaminated salivary sample, as the salivary cortisol to cortisone ratio was concerning for contamination with exogenous steroid (1)

Discussion:

Work up for Cushing's syndrome can be very confusing and frustrating at times for the patient and their physicians. Doing a meticulous work up is necessary to reach an accurate conclusion. Misdiagnosing Cushing's can lead to a

cascade of mistreatment with serious consequences. The case presented highlights the challenges encountered in taking care of such patients. It is necessary to understand the pre-testing probability to reach a precise conclusion. Factitious disorder or sample contamination can be yet another challenge in the differential diagnosis of Cushing's work up.

(1)
Raff H Measurement of Late-Night, Salivary Cortisol and Cortisone by LC-MS/MS to Assess Preanalytical Sample Contamination with Topical Hydrocortisone. Clinical Chemistry 58:5 (2012)

Reproductive Endocrinology

CLINICAL STUDIES IN FEMALE REPRODUCTION I

Comparison of Estradiol by Mass Spectrometry Versus Immunoassay in Women Undergoing Menopause: Study of Womens Health Across the Nation (SWAN)

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Serum estradiol (E2) concentrations in midreproductive women are easily measured using a variety of conventional immunoassays (IA). However, when women approach and traverse menopause, E2 eventually drops below levels where IA lacks sufficient sensitivity to accurately measure E2. Liquid chromatography and tandem mass spectrometry (LC/MS/MS) has become the standard method for assessing steroid hormones, especially when circulating concentrations are low. We evaluated the relationship between IA and LC/MS/MS E2 measurements in a cohort of women taken from the Study of Womens Health Across the Nation (SWAN) to assess the degree of agreement between the two methods and to determine the level of E2 at which IA becomes unreliable.

Methods: 315 serum samples that had been previously measured for E2 using IA were re-analyzed using LC/MS/MS performed by one of the authors (RA). In this original set, E2 levels that were below the limit of assay detection (LLD, 6 pg/ml) were interpolated as a random number between 0 and the LLD. Agreement between all 315 samples was assessed using both Pearson and Spearman correlation. The analysis was repeated excluding the subset of specimens that were below the lower limit of detection (LLD) for the IA E2 assay (6 pg/ml; N=176), and a third set of correlations was obtained for specimens that measured <15 pg/ml by IA but were above the 6 pg/ml LLD (N=82).

Results: The overall dataset (N=315) demonstrated excellent agreement between IA and LC/MS/MS with a Pearson's r and Spearmans r of 0.98 AND 0.60, respectively. When the subset of 176 samples above the LLD were assessed, Pearson's r was 0.98 and Spearman's r was 0.81. In contrast, when specimens measuring 6–15 pg/ml by IA were compared to LC/MS/MS, Pearson's r was -0.03 and Spearman's r was 0.09, indicating a complete loss of relationship between the two methods.

Conclusions: The IA used by SWAN (England, Clin Chem 2002; 48: 1584) and LC/MS/MS demonstrate excellent correlation for E2 measurements above 15 pg/ml. However, circulating concentrations of E2 below 15 pg/ml were not accurately measured using IA.

Reproductive Endocrinology

FEMALE REPRODUCTION: BASIC MECHANISMS

NALCN Expression Is Regulated by Progesterone and Estrogen in Human Myometrial Smooth Muscle Cells

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During pregnancy, the uterus transitions from a quiescent state to a highly contractile, excitable state. Both ion channels and hormones are essential for this transition. We recently identified that the Na^+ leak channel, non-selective (NALCN) contributes to a leak current in human MSMCs and mice lacking NALCN have prolonged and dysfunctional labor. Additionally, NALCN levels change throughout mouse pregnancy suggesting regulation by hormones of pregnancy, specifically estrogen and progesterone. Here, we tested the hypothesis that P4, a pro-quiescent hormone, and E2, a pro-contractile hormone, regulate NALCN expression and current in the myometrium. In a human immortalized myometrial cells (HM6ERMS2), using qPCR we measured a 2.3 fold decrease and a 5.6 fold increase in NALCN mRNA expression in the presence of E2 and P4, respectively. These findings were also confirmed when NALCN protein expression were measured by immunoblot. Conversely, treatment with the ER antagonist, ICI 182,780, significantly increased NALCN mRNA expression, while treatment with the PR antagonist RU486 significantly decreased NALCN mRNA expression suggesting E2 and P4 work through their respective receptors to regulate NALCN. P4 differentially regulates myometrial activity depending on which progesterone receptor is activated: PRA, promotes contractility, whereas PRB promotes quiescence. Thus to study the effect of each PR, we used a human myometrial cell line stably expressing PRA or PRB, and measured similar increases in NALCN mRNA expression in both cell lines treated with P4. To determine the functional consequences of E2 and P4, we measured NALCN-dependent leak current in MSMCs using whole cell patch clamping. We observed that E2 significantly inhibited while P4 significantly enhanced NALCN current. Finally, we identified estrogen response and progesterone response elements (ERE and PRE) in the NALCN promoter and showed that the PREs contributed to P4 regulation while the ERE did not contribute to the regulation of NALCN expression using luciferase based promoter assays. Overall, our findings show that NALCN is upregulated by P4, the