Detection of miRNAs in HPA axis function in healthy subjects

Background

Cortisol is a steroid hormone involved in metabolism, inflammation and stress response (1). It is secreted by the adrenal glands in a pulsatile fashion in response to a feedback loop involving the hypothalamus-pituitary-adrenal (HPA) axis. Corticotropin-releasing hormone (CRH) is secreted from the hypothalamus in response to circadian rhythm changes and in response to stressors. CRH stimulates corticotroph cells in the anterior pituitary gland to secrete adrenocorticotropic hormone (ACTH), which, in turn, promotes synthesis of corticosteroids such as cortisol in the adrenal glands. Secretion of cortisol from the adrenal gland then downregulates hypothalamic CRH and pituitary ACTH secretion through feedback inhibition.

Cortisol stimulation is used to assess the adequacy of HPA axis functional response in humans. However, accurate cortisol measurements can be misleading, especially in the presence of pituitary and hypothalamic diseases affecting HPA axis response. New biomarkers for HPA axis function are needed.

We recently showed significant changes in miRNA expression profiles in the serum of humans and mice during HPA axis regulation and identified miRNA that might be useful as biomarkers of HPA axis function. We found that miRNA levels are significantly decreased 30 minutes after ACTH stimulation in mice and humans, while dexamethasone increases serum miRNAs in mice.

The proposed study will test dexamethasone effect on miRNA profile in healthy humans. As our data indicate that miRNA levels change over time, we will study the time course of miRNA response to 1 mg IV dexamethasone within 60 minutes after injection.

Dexamethasone is used routinely in human subjects for the treatment of inflammatory conditions and as a diagnostic tool in the evaluation of excess cortisol secretion (Cushing's syndrome). For the latter, 1 mg dexamethasone is given orally at midnight. However, oral formula absorption in the gut can range from 20 to 60 minutes due to the presence of food and other factors. IV administration will bypass this variability and ensure an accurate and reproducible time-course study.

We will compare miRNA expression before and after dexamethasone treatment. We will follow miRNA expression dynamics over a 1-hour time course to identify peak expression levels and to correlate miRNA expression with circulating dexamethasone levels.

Dexamethasone and miRNA levels will be checked at baseline just before injection and then at 15, 30, 45, and 60 minutes after injection. Dexamethasone level is taken to verify treatment and to correlate it with miRNA expression.

Definitions

- **miRNA** (also called microRNA or miR), small sequences of RNA that bind and inhibit specific areas in the gene and regulate gene expression. Most of the miRNAs are intracellular, but some can be found in the circulation.
- **mRNA** (messenger RNA), a nucleic acid polymer molecule transcribed from DNA that serves as a template for the synthesis of the specified protein. The level of mRNA indicates changes in gene expression.
- **Adrenal gland,** an endocrine gland located above the kidney. It produces multiple steroid hormones, most importantly cortisol (or corticosterone in rodents) and aldosterone. Its inner core produces epinephrine and norepinephrine.

- **Serum,** the liquid portion of the blood after it has been allowed to clot. It is free of cells and platelets.
- **Plasma**, the liquid portion of blood that has been prevented from clotting. It includes cells and WBC can be extracted from it.
- **Dexamethasone**, a steroid hormone that belongs to the glucocorticoid steroid hormone group that also includes cortisol. It binds specifically to the glucocorticoid receptor to induce cellular and tissue outcomes similar to those seen with cortisol. It is use clinically to reduce inflammation and as a diagnostic tool for Cushing's syndrome.
- **Cushing's syndrome**, a disorder caused by increased production of cortisol. Symptoms include increased appetite and weight gain, mood changes, trouble sleeping, nausea, upset stomach, easy bruising, swelling, hypertension, hyperglycemia, osteoporosis.

Study Protocol

Subjects

Twenty subjects will be recruited from CSMC employees. No information will be gathered from subjects' medical files. An email will be sent out to all members of the Endocrinology department, the Pituitary Center, and affiliated offices (physicians, scientists, technicians, nurses, administrators). Employee email addresses will be included as BCC so as not to reveal the distribution list. In this email, study staff will detail all planned procedures, inclusion/exclusion criteria, and potential side effects. Those who express interest in participating will be contacted by study staff, who will review with them the protocol and informed consent and confirm eligibility for enrollment. Study staff will not enroll subjects who directly report to them to mitigate the potential for coercion.

Inclusion Criteria

- Adults age ≥ 18

Exclusion Criteria

- Pregnancy
- Presence of a disease that affects HPA axis function
- Current use of medications that affect cortisol secretion, such as opioid pain medication, anti-fungal agents, anti-epileptic agents, and glucocorticoids, including hydrocortisone, prednisone, dexamethasone, steroid-containing inhalers, and steroid injected into the joints
- History of adverse event related to use of glucocorticoids

Procedures

- All procedures will be performed at the CTRC by staff nurses.
- A vein line will be inserted to allow for dexamethasone injection and for easier blood draws during the study.
- A total of 60 mL will be taken from each subject, drawn into 10 red-cap tubes (6 mL each) at 5 time points over the course of the study.

- At baseline, before dexamethasone injection, blood will be drawn into 2 red cap tubes: one tube will be used to assess dexamethasone levels and one tube will be used to assess for miRNAs.
- Immediately after baseline blood collection, 1 mg dexamethasone will be administered intravenously at a rate of 4 mg/min.
- After injection, blood will be drawn at 15, 30, 45, and 60 minutes into 2 red cap tubes: one tube will be used to assess dexamethasone levels and one tube will be used to assess for miRNAs.
- After the 60-minute blood draws, the IV line will be removed, and the subject will be free to leave the facility.

Adverse Effects

We have extensive experience with the oral dexamethasone 1 mg diagnostic test for Cushing's syndrome. We expect no new side effects with this same single dose administered intravenously. Side effects commonly associated dexamethasone are observed with higher doses and longer use of this medication. These effects mimic symptoms of Cushing's syndrome and include swelling, increased appetite, weight gain, mood changes, trouble sleeping, nausea, upset stomach, easy bruising, hypertension, and hyperglycemia.

We expect no adverse events from the blood draws.

Blood Processing

- Red-cap tubes used to collect blood to assess dexamethasone levels will be sent to the CSMC Quest laboratory.
- Red-cap tubes used to collect blood to assess for miRNAs will be put on ice and hand-delivered by study staff to Dr. Shlomo Melmed's laboratory (Davis 3024) for serum extraction.
    - Serum will be stored in -80°C freezer.
    - Analysis of miRNAs will be done as one batch on all samples collected.

Data Collection and Storage

- Subject name, age, and sex will be recorded by study staff.
- At CTRC, blood draw tubes will be coded using the subject's initials and the day of collection. For example, blood from John Adam Smith collected on April 24, 2018, would be coded as J04A24S18. If the middle name is not available, there will be no letter in the fourth position.
- This same code will also be used during sample processing and analysis.
- Subjects will be identified only on the PI's personal computer at CSMC.
- Data will be stored for 5 years and will be accessible only to the PI.

Compensation

Subjects will be compensated with a $25 Target gift card.

REFERENCES

1. Stewart P, Newell-Price, JDC. The adrenal cortex. In: Melmed S, et al, eds. *Williams Textbook of Endocrinology*, 13th ed. Philadelphia, PA: Elsevier; 2016.
2. Yamamoto M*, Ben-Shlomo A*, Kameda H, et al. Somatostatin receptor subtype 5 modifies hypothalamic-pituitary-adrenal axis stress function. *JCI Insight.* 2018;3:e122932. *Equal contribution.
3. Altuvia Y, Landgraf P, Lithwick G, et al. Clustering and conservation patterns of human microRNAs. *Nucleic Acids Res*. 2005;33:2697-2706.