The Use of Salivary Cortisol Measurement by Mass Spectrometry to Identify Low Serum Cortisol Values in Healthy Volunteers Receiving CRN04894, A Melanocortin 2 Receptor (MC2R) Antagonist

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BACKGROUND AND OBJECTIVES

- Clinical trials of cortisol-lowering drug candidates would benefit from user-friendly methods to measure cortisol in an outpatient setting.
- CRN04894 is a potent, orally bioavailable, MC2R antagonist in development for the treatment of adrenocorticotropic hormone (ACTH)-dependent Cushing's syndrome and congenital adrenal hyperplasia.
- We have previously reported results from a randomized, double-blinded, placebo-controlled (6 active:3 placebo/cohort), multiple (10-day) ascending dose (40 to 80 mg/day) study in healthy volunteers.[1]
- Dose-dependent declines in basal and ACTH-stimulated serum cortisol levels and a median reduction of 75% in 24-hr urinary-free cortisol (UFC) were seen in the 80 mg cohort. Asymptomatic glucocorticoid deficiency (defined as 08:00 serum cortisol of <5 µg/dL) was the most commonly observed adverse event.[1]
- Here, we report:
  - The relative changes in serum, saliva and urinary cortisol with CRN04894 therapy.
  - The time scale of changes in measures of disease activity.
  - The use of salivary cortisol to diagnose glucocorticoid deficiency.

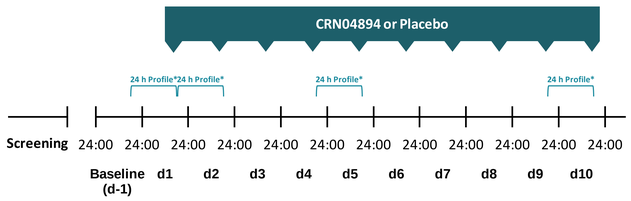
1. A. Krasner et al. Inhibition of Basal and ACTH-stimulated Cortisol Secretion in Humans Using an Oral, Nonpeptide ACTH Antagonist (CRN04894). Oral presentation at ENDO 2022.

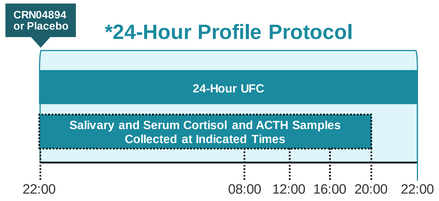
STUDY DESIGN AND METHODS

- We evaluated cortisol and ACTH data from the three cohorts of healthy volunteers dosed once daily for 10 days at 22:00 with either 40, 60, or 80 mg or placebo.
- Salivary and serum Cortisol Day Curves (CDC) and ACTH day curves (results reported as the mean of 4 or 5 available samples collected at 22:00, 08:00, 12:00, 16:00 and 20:00) studies were undertaken on days -1, 1, 4, and 9.
- Any subject with a 08:00 serum cortisol of <5 ug/dL was commenced on hydrocortisone (10 mg at 08:00, 5 mg at 14:00) 'add-back'.
- Data from subjects with Covid-19 and samples from subjects receiving 'add-back' hydrocortisone were excluded.

Assays

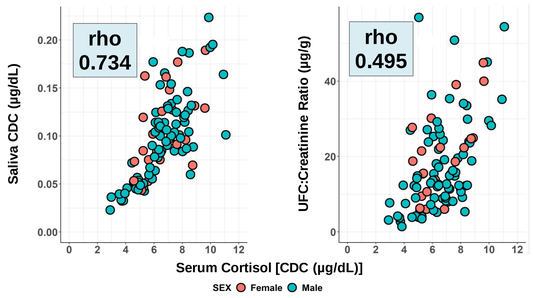
- Serum, saliva and urinary cortisol was measured using a "fit-for-purpose" validated LC-MS/MS method (Sciex 5500 LC-MS/MS system consisting of a Shimadzu LC and chromatographic separation with a Waters UPLC BEH C18 1.7 µm (2.1 x 50 mm) column). The lower limit of quantification was 0.005 µg/dL in saliva, 0.01 µg/dL in serum and urine.
- Plasma ACTH was measured on a bead-based Luminex MAGPIX® sandwich immunoassay using MILLIPLEX® MAP Kit (EMD Millipore Corporation: #HPTPMAG-66K).





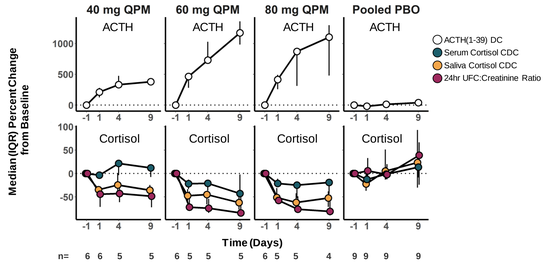
RESULTS

Mean Serum Cortisol Was Strongly Correlated with Mean Salivary Cortisol and Moderately Correlated with 24-hr UFC:Creatinine Ratio



- Correlations were not different for females and males.

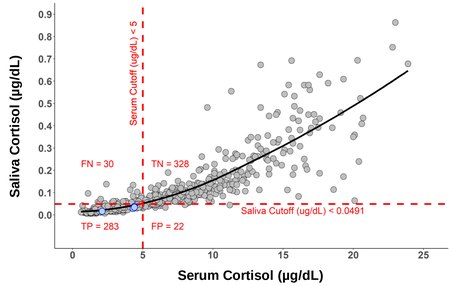
CRN04894 Competes with ACTH(1-39) and Reduces Cortisol



Subject data without all 3 cortisol analytes at a visit excluded

- Cortisol reductions were largely achieved within first day of treatment and were sustained.
- Favorable CRN04894 competition of ACTH was maintained despite expected increase in ACTH from attenuated cortisol negative feedback.
- Consistent magnitude ranking for change in measured cortisol analytes: serum < saliva < urine.

Saliva Provides a Non-Invasive Cortisol Threshold to Confirm Need for Glucocorticoid Rescue



Individual time-matched saliva and serum cortisol values from all visits from Day -1 to study completion.
Black line is loess fit for trend visualization purposes.
Concentrations below the lower limit of quantification (BLQ) were excluded from this analysis.

- A saliva cutoff for cortisol deficiency was optimized for diagnostic accuracy (92.2%) using a serum cortisol value of <5 µg/dL as the reference.
- Four subjects had 08:00 serum cortisol <5 µg/dL measured on a day when saliva cortisol was simultaneously collected. All four were true positives ( ) based on the saliva cortisol cutoff of <0.0491 µg/dL.

CONCLUSIONS

- CRN04894 effectively lowered cortisol measured in serum, saliva, and urine in healthy volunteers, despite compensatory rises in endogenous plasma ACTH.
- Serum and salivary cortisol day curves were strongly correlated.
- A low salivary cortisol measured by mass spectrometry (<0.0491 ug/dL) was highly predictive of a low serum cortisol (<5 ug/dL). These data suggest that saliva samples collected out-of-hospital may be of value in the retrospective confirmation or exclusion of suspected glucocorticoid deficiency.
- Future studies will explore the use of salivary cortisol and cortisone measurement in patients with ACTH-dependent Cushing's syndrome receiving CRN04894.

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A. Krasner et al., 2022