available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/steroids

Use of steroid profiles in determining the cause of adrenal insufficiency

Jennifer P. Holst[a], *Steven J. Soldin*[b,c], *Rochelle E. Tractenberg*[d], *Tiedong Guo*[b,e], *Priya Kundra*[f], *Joseph G. Verbalis*[f], *Jacqueline Jonklaas*[f,*]

[a] *Center for Diabetes and Endocrinology, University of Pittsburgh Medical Center, Pittsburgh, PA, USA*
[b] *Bioanalytic Core Laboratory, General Clinical Research Center, Georgetown University Medical Center, Washington, DC, USA*
[c] *Department of Laboratory Medicine, Children's National Medical Center, Washington, DC, USA*
[d] *Departments of Neurology, Biostatistics, Bioinformatics & Biomathematics, and Psychiatry, Georgetown University Medical Center, Washington, DC, USA*
[e] *Department of Biology, The Catholic University of America, Washington, DC, USA*
[f] *Division of Endocrinology, Georgetown University Medical Center, Washington, DC, USA*

ARTICLE INFO

Article history:
Received 27 August 2006
Received in revised form
27 October 2006
Accepted 1 November 2006
Published on line 8 December 2006

Keywords:
Adrenal insufficiency
Steroid profile
Tandem mass spectrometry

ABSTRACT

Hypothesis: A cortisol response to adrenocorticotropin injection is the standard test for diagnosing adrenal insufficiency. Multiple steroid hormones can now be accurately measured by tandem mass spectrometry in a single sample. The study objective was to determine whether a steroid profile, created by simultaneous measurement of 10 steroid hormones by tandem mass spectrometry, would help determine the cause of adrenal insufficiency.
Design: A 10-steroid profile was measured by tandem mass spectrometry during the performance of a standard high dose cortrosyn stimulation test. The steroids were measured at baseline, 30, and 60 min following synthetic adrenocorticotropin injection. Adrenal insufficiency was defined as a peak cortisol level of less than 20 μg/dL. Testing was conducted in the general clinical research center of a university medical center. Normal volunteers, patients suspected of having adrenal insufficiency, and patients with known adrenal insufficiency participated.
Results: Our results showed that adrenal insufficiency of any cause was adequately diagnosed using the response of 11-deoxycortisol, dehydroepiandrosterone, or these analytes combined in a two-steroid profile. A three-steroid profile yielded a test with 100% accuracy for discriminating primary adrenal insufficiency from normal status. Primary adrenal insufficiency was well separated from secondary adrenal insufficiency using only a single aldosterone value. 11-Deoxycortisol, dehydroepiandrosterone, and a two-steroid profile each provided fair discrimination between secondary adrenal insufficiency and normal status.
Conclusions: We conclude that stimulated levels of aldosterone, 11-deoxycortisol, dehydroepiandrosterone, and a two- or three-steroid profile provided additional discrimination between states of adrenal sufficiency and insufficiency. It is proposed that a steroid profile measuring cortisol, aldosterone, 11-deoxycortisol, and dehydroepiandrosterone would potentially improve the ability to determine the cause of adrenal insufficiency.

* *Corresponding author at*: Division of Endocrinology and Metabolism, Georgetown University Hospital, Suite 232, Bldg. D, 4000 Reservoir Road, NW, Washington, DC 20007, USA. Tel.: +1 202 687 2818; fax: +1 202 687 2040.
E-mail address: jj@bc.georgetown.edu (J. Jonklaas).
0039-128X/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.steroids.2006.11.001

1. Introduction

A deficient steroid hormone response to adrenocorticotropin (ACTH) is a hallmark of adrenal insufficiency (AI) [1]. Measurement of serum cortisol levels following synthetic ACTH injection forms the traditional "gold standard" for diagnosing AI [1–3]. A decreased cortisol response is seen in both primary and chronic secondary AI [4], and diagnostic "cut-off" values of 18–20 μg/dL are well-documented [2,3,5–14]. Aldosterone levels after ACTH stimulation can be helpful to distinguish between primary and secondary AI. Despite the wealth of data regarding ACTH-stimulated cortisol levels, studies of aldosterone responses to ACTH stimulation in normal and adrenally insufficient subjects are few [9,12,15]. Thus, the stimulated aldosterone level appropriate for distinguishing between primary and secondary adrenal insufficiency is uncertain.

There has been interest in using the levels of steroids other than cortisol and aldosterone to discriminate normal adrenal function from AI, particularly in situations where the cause of AI cannot readily be established. These steroid hormones include 11-deoxycortisol [7,13], 17-hydroxyprogesterone [16], progesterone, androstenedione, dehydroepiandrosterone (DHEA) [16], and dehydroepiandrosterone-sulfate (DHEA-S) [17,18]. However, responses of these other steroid hormones to ACTH stimulation have not been frequently studied.

Immunoassay is a sensitive method for measuring steroid hormones [19]. However, testing programs show lack of specificity in the commercially available immunoassays [20,21]. Tandem mass spectrometry (MS/MS) has been shown to have better specificity than immunoassay [22], and has been used to measure cortisol during an ACTH stimulation test [23]. We developed a method to simultaneously measure nine steroid hormones by atmospheric pressure photoionization ion source MS/MS and have shown this method to be highly specific and sensitive [24,25]. These nine hormones are cortisol, 11-deoxycortisol, 17-hydroxyprogesterone, progesterone, androstenedione, DHEA, DHEA-S, estradiol, and testosterone. An additional refinement allows simultaneous of twelve steroids including aldosterone [26].

In this study we measured 10 adrenal hormones in normal subjects and subjects with AI. The first study aim was to assess whether any steroids other than cortisol increased significantly in response to an ACTH stimulus. The second study aim was to determine the correlation between peak cortisol levels and the peak levels of other steroids. We were interested in whether a profile combining some of the other correlating steroids would also be helpful in determining the cause of AI, or in making a diagnosis of AI, particularly in borderline or clinically difficult cases. The final study aim was to generate additional data regarding aldosterone values in normal subjects and subjects with AI.

An additional topic that is not examined in this analysis, but that is being addressed in a separate manuscript is the performance of cortisol immunoassays compared with MS/MS assays. This analysis examines the issue of whether patients are differently classified with respect to their adrenal status using the two assays and a cortisol cut-off of 20 μg/dL or above. This will allow validation of MS/MS assays and their comparison with existing "gold standard" methods.

2. Methods

2.1. Subjects and protocol

Normal volunteers, patients suspected of being adrenally insufficient who required an ACTH stimulation test for routine clinical evaluation, and patients with known adrenal insufficiency were recruited. The Institutional Review Board approved the study protocol. Normal subjects who were pregnant or breast-feeding and patients with diabetes were excluded from participation. Following informed consent, subjects underwent a standard high-dose ACTH stimulation test performed on an outpatient basis in the general clinical research center. Patients were in a seated position and rested for at least 10 min before testing began. A 23-gauge indwelling catheter was inserted and a baseline blood sample was drawn. Following injection of 250 μg Cortrosyn™ blood samples were taken at 30 and 60 min. Blood pressure and heart rate were measured before and after testing. A medication history was taken and only patients with known AI were taking steroid-containing medications. On the test day patients with known AI delayed their glucocorticoid and mineralocorticoid replacement until testing was complete. Information regarding dietary salt intake was not collected.

2.2. Laboratory measurements

At all three-time points, 10 steroids were analyzed by MS/MS as previously described [24–26]. Serum samples were deproteinized and injected onto the column after centrifugation. Chromatographic separation was carried out on a reverse phase C-18 [25] or C-8 [26] analytic column. Samples were washed online and eluted using a methanol–water gradient and introduced into the mass spectrometer. The current assay protocol [26] separates the total ion chromatogram into four sections in order to optimize parameters for each individual analyte. Atmospheric pressure photoionization was used to measure nine of the steroids in the positive ion mode; aldosterone was measured in the negative mode. The method is based on isotope dilution and is specific for the steroid of interest. Aldosterone was analyzed after adding an aliquot of cortisol to prevent precipitation of aldosterone with cortisol-binding globulin. There was less interference with neighboring peaks when aldosterone was separated from other steroids in the negative ion mode in a separate time period. This assay has no cross-reactivity with fludrocortisone. The API-3000 or API-5000 mass spectrometer was used for all assays. Our first generation steroid profile assay required 760 μL of serum and measured 9 steroids in 18 min [25]. Our second generation assay uses 200 μL of serum and measures 12 steroids in 11 min [26]. Assay accuracy was evaluated in two ways. The assays were compared with MS/MS assays performed at the Mayo Clinic: correlation coefficients ranged from 0.908 to 0.999 [26]. Recovery studies were also performed and yielded mean recoveries of the analytes of 90–110% [26].

Patients suspected of having AI also had cortisol levels determined by immunoassay in a clinical laboratory; these results were used to guide clinical decision-making. However, for the purposes of this analysis a peak cortisol concentration