THE ANALYSIS OF THE EMBEDDED CORTISOL SIGNATURE IN THE NAIL OF THE DOMESTIC DOG *(Canis lupus familiaris)*

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Abstract

Cortisol is a glucocorticoid steroid hormone that is released from the adrenal gland in response to stress in most mammal species; other vertebrates use the structurally similar corticosterone. There is currently no method to measure cortisol levels from the nail of an animal, which would allow a more accurate analysis of long-term cortisol secretion in relation to current techniques such as measures of blood and saliva. In this study, we proposed a novel technique to analyze cortisol within the nail of the domestic dog *(Canis familiaris)*. Nail was homogenized through exposure to liquid nitrogen and use of an attrition mill. Cortisol was then recovered through solid phase extraction and recovery was determined through enzyme-linked immunoassay. We have found that cortisol is present within the nail at a measurable level. Validation of this technique is currently being completed to determine effectiveness as a diagnostic tool. This research will lay the groundwork for advancing technology available surrounding cortisol testing.

Introduction

The endocrine system is a vital component of animal physiology that regulates growth and development, along with coordinating responses to environmental stimuli. Cortisol is a glucocorticoid steroid hormone that is secreted by the adrenal gland in response to a stressor. Chronic exposure to cortisol can suppress the immune system, growth, development, reproduction, and metabolism. There is currently no method to measure cortisol levels from the nail of an animal, which could allow for an accurate analysis of long-term cortisol secretion, as compared to current techniques. Currently used blood and saliva testing do not give a clear indication to long-term secretion and are often influenced by the current environmental conditions or the normal daily rhythms of cortisol secretion. Here we describe the methodology for extracting and measuring cortisol for the dog nail.

Methods

1. Homogenization of Tissue

Nail samples from 23 dogs were collected by the staff at Loch Haven Veterinary Hospital, Winter Park, FL. Nail samples were homogenized to prepare them for cortisol extraction through either a bead homogenizer or the use of a hand-held attrition mill.

2. Solid Phase Extraction

Steroid was extracted and purified from nail samples using solid-phase extraction. Methanol was used to elute impurities from the sample and retain the steroid product in the stationary sorbent. Cortisol was recovered with a final elution of 90% methanol.

3. Cortisol Enzyme-linked Immunoasssay

Levels of cortisol were analyzed through cortisol enzyme-linked immunoassay from Arbor Assays, and determined through interpolation of the generated standard curve. The samples were read on a plate reader at 495 nm wavelength and data was analyzed using GraphPad Prism 6.

Results

Table 1 – Mean cortisol concentrations from a common pool recovered from different methods of nail homogenization. Numbers in parentheses indicate sample size.

Selected Tube	Selected Beads	Mean Cortisol Concentration (ng/g) ± SE
Stainless Steel with Liquid Nitrogen	6 Zirconia Beads (3)	1.27 ± 0.76
Stainless Steel	6 Zirconia Beads (6)	1.36 ± 0.52
Stainless Steel	4 Chrome Steel Beads (3)	1.82 ± 0.94
Reinforced Plastic with Liquid Nitrogen	6 Zirconia Beads (3)	0.42 ± 0.19
Reinforced Plastic	6 Zirconia Beads (2)	4.37 ± 3.58
Hard Tissue Lysing Kit	Zirconia Oxide Beads (3)	1.87 ± 0.90
Hard Tissue Lysing Kit Washed With Ethanol	Zirconia Oxide Beads (1)	1.63
Ethanol Wash	No beads (2)	0.20 ± 0.01

Table 2 – Determination of percent steroid recovery in cortisol spiked samples from a sample pool (n = 10).

Concentration Spike (ng/g)	% Recovery
113	93
22	70
13	1.7
6	0.1

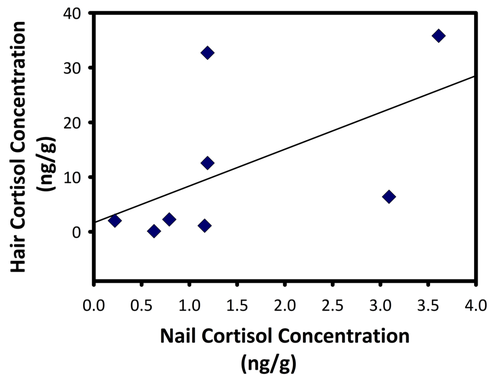

Figure 1 – Correlation between cortisol concentration in nail and hair analysis. Concentration values were interpolated from a standard curve generated in GraphPad Prism 6. Samples had a correlation value of 0.556 (n = 8).

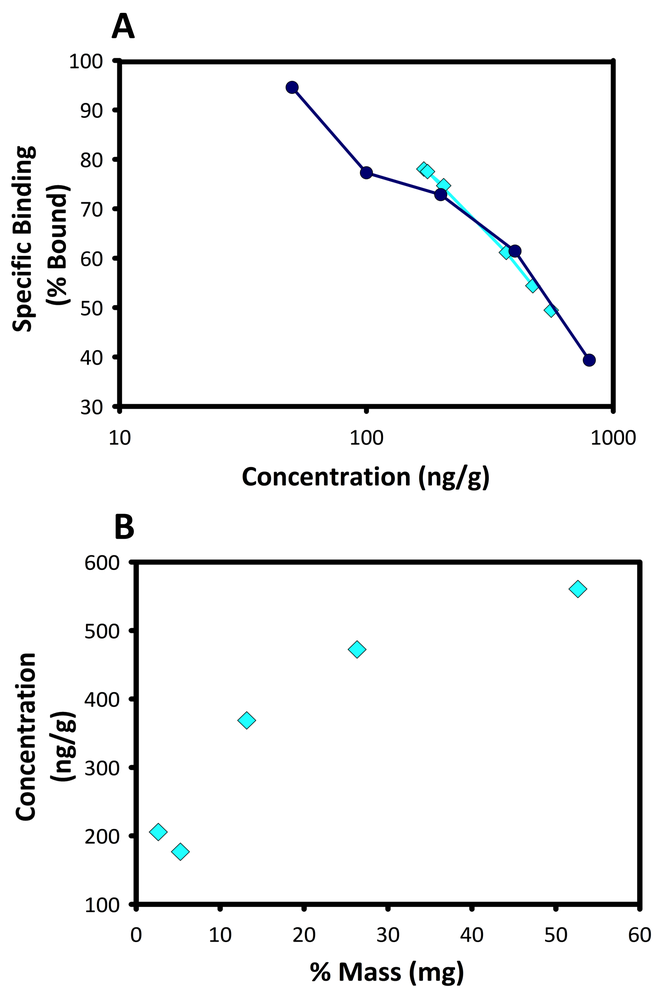

Figure 2 – Cortisol concentrations of a serial dilution of cortisol sample pool. Concentration values were interpolated from the standard curve generated in GraphPad Prism 6. (2A) – Cortisol concentrations of the sample pool at points of specific cortisol binding. **Circular** points comprise the standard curve, while diamond represents individual nail samples from the pool. Samples were within linear range of and parallel to the standard curve ($R^2 = 0.982$, $P < 0.05$, n = 10) , thus suggesting no substances interfering in the nail matrix. (2B) – Cortisol concentrations of the sample pool in comparison to the percent mass (n = 10).

WHAT IS NEXT? This research will be further explored through continued validation of technique with analysis of individual nail and hair samples. The use of liquid chromatography and its utility in recovering the steroid product will also be investigated.

Discussion

Using the nail as a biomarker for estimating patterns of long-term cortisol secretion may allow for a variety of future applications. Nails are easily clipped without negatively affecting appearance, which has been a concern of similar diagnostics using hair. The collection of nails is much less invasive than the collection of blood or saliva and does not require additional timing or restraint. Beyond use in the veterinary industry, cortisol could be analyzed in wild mammals and even potentially extinct species to gather information surrounding the long-term stress. This information may also be relevant in determining an animal's physiological state in legal cases surrounding animal neglect and abuse.

Acknowledgments

•The authors would like to thank **Dr. Jim Martin** and staff for his continued provision of nail samples from Loch Haven Veterinary Hospital.

• This research was funded through the **Edward W.** and **Stella C. Van Houten Memorial Fund**, **the Office of the Dean of Arts and Sciences,** and startup funds provided by the Department of Biology.