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Dehydroepiandrosterone Sulfate in Dogs with Hypercortisolism

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Abstract: *Hyperadrenocorticism (HAC) is characterized by an increase the secretion of cortisol and/or other hormones secreted by the adrenal cortex. Dehydroepiandrosterone sulfate (DHEA-S) is a steroid precursor synthesized from cholesterol; its evaluation in dogs with HAC receiving trilostane treatment may provide important information about the disease. This study assessed DHEA-S concentrations in dogs with ACTH-dependent HAC treated with trilostane to establish an alternate method of HAC diagnosis. Twenty-five dogs were divided into: group A (n = 6) included healthy dogs with no evidence of systemic disease; group B (n = 10) included dogs diagnosed with ACTH-dependent HAC; and group C included dogs with HAC treated with trilostane (n = 9). The dogs treated with trilostane had higher mean DHEA-S values than healthy dogs or dogs with untreated HAC. This result demonstrated the enzyme inhibition by trilostane in the cortisol and sexual hormone pathway.No significant differences were observed in the mean DHEA-S values among dogs in groups A and B, hence DHEA-S concentrations could not distinguish healthy animals from dogs with untreated HAC. We conclude that the measurement of DHEA-S is not a reliable method for the diagnosis of CAH, however other studies should be performed to elucidate its role in the therapeutic monitoring of dogs with CAH and to correlate their values with cortisol values.*

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I. Introduction

Dehydroepiandrosterone sulfate (DHEA-S), a dehydroepiandrosterone metabolite, is a steroid precursor synthesized from cholesterol and released by the zona fasciculata, and in smaller amounts by the gonads and brain. In humans, its half-life is approximately 1–3 hours. For this reason, DHEA-S is a stable product that can be used for evaluation, as there are no fluctuations in measurement.[4,6]

Cholesterol is enzymatically transformed into pregnenolone and then into 17-hydroxypregnenolone; after transformation it is converted to DHEA and DHEA-S. The latter dissociates into dehydroepiandrosterone before being transformed into the androgens androstenedione and testosterone and the estrogen estradiol.[5]

DHEA-S is synthesized in response to ACTH in humans,[8,11]squirrels,[4] and dogs.[9] DHEA-S has already been assessed in humans with hyperadrenocorticism (HAC), as the response to ACTH is well established in this species. This hormone is a promising marker to monitor HAC patients who undergo transsphenoidalhypophysectomy, as the blood levels increase early in patients with disease recurrence.[6]

HAC is one of the most common endocrine diseases in dogs, and is characterized by excessive secretion of glucocorticoids when the spontaneous form occurs.[2,15]The gold standard for diagnosing HAC is the low-dose dexamethasone suppression test. However, ACTH stimulation test should be performed to confirm the increased production of cortisol in cases negative results are obtained at suppression tests.[2]

The treatment of choice for ACTH-dependent HAC is trilostane, a competitive inhibitor of 3-beta-hydroxysteroid dehydrogenase enzyme (3β-HSD) responsible for the conversion of DHEA into androstenedione and 17-hydroxypregnenolone into 17-hydroxyprogesterone, which are essential steps for cortisol production. Inhibiting the isomerase system (3β-HSD) results in decreased production of cortisol even with excessive ACTH stimulus.[16] In accordance with studies[19], dogs treated with trilostane had increased 17-hydroxypregnenolone and DHEA, confirming the inhibitory effect of trilostane on the 3β HSD enzyme.

The aim of this study was to evaluate the serum levels of DHEA-S in dogs with spontaneous hypercortisolism at the time of diagnosis and during treatment with trilostane in order to understand the dynamics of this hormone in dogs with spontaneous HAC.

II. Material And Methods

The experimental protocol used in this study was approved by the Ethics Commission of Animal Use (CEUA) of the FCAV/UNESP, Campus of Jaboticabal-SP (protocol 6.367/16).

This is a prospective study and the dogs included in this study were treated at the Veterinary Teaching Hospital of the Faculty of Agrarian and Veterinary Sciences, Paulista State University "Júlio de MesquitaFilho", Jaboticabal, Brazil, from December 2013 to February 2015 under the owner's authorization. Adult dogs 7–15 years of age, regardless of sex or breed, were selected for this study.

The dogs were divided into three groups as follows: healthy dogs (group A, n = 6), dogs diagnosed with HAC (group B, n = 10) and dogs with HAC treated with trilostane (group C, n = 9). Group A was the control group; samples were collected from dogs at the kennel of the Veterinary Teaching Hospital that showed no signs of disease. To confirm the absence of HAC, these dogs underwent the ACTH stimulation test (Synacthen 0.25 mg/mL, Biofutura Pharma, Italy) (5 µg/kg intravenous [IV]), authorized by the local CEUA under protocol no. 023938/14.

Group B samples were collected from dogs that initially presented with clinical signs suggestive of spontaneous HAC, such as polyuria, polydipsia, polyphagia, hyperlipidemia, telangiectasia, cutaneous calcinosis, and/or obesity, and that had HAC confirmed by analysis of cortisol levels before and after the ACTH stimulation test (5 µg/kg IV). Sub-classification into ACTH-dependent HAC was dependent on bilateral enlargement of the adrenal glands and absence of images suggestive of neoplasia through an abdominal ultrasound, as described previously[15].

Group C samples were collected from dogs diagnosed with HAC and treated with trilostane without any clinical sign of the disease. The mean trilostane dose was 2.5 mg/kg PO SID (2–4 mg/kg) and the mean treatment duration was 90 days (60–140 days). Samples were collected after the ACTH stimulation testwas performed for HAC monitoring. In these cases, only post-ACTH cortisol was measured.[3]

Cortisol measurements were performed by a commercial laboratory (BET Laboratories, Rio de Janeiro, RJ, Brazil) using a radioimmunoassay technique (MP Diagnostics Cortisol® kit, USA). Reference values considered in this study were those adopted from the same laboratory used to diagnose HAC (pre-ACTH cortisol, 5–60 ng/mL; post-ACTH cortisol, 60–170 ng/mL); the values were different for dogs treated with trilostane (post-ACTH cortisol, 20–50 ng/mL).

For DHEA-S dosing, serum samples were stored at -80ºC until use; simultaneous measurement was performed using an ELISA kit commercial (Human Diagnostics®, catalog n. 55060, Germany) according to the manufacturer's instructions and as standardized previously.[18] DHEA-S concentrations were quantified in basal or post-ACTH samples of groups A, B, and C. In a similar experimental protocol, DHEA-S concentrations remained stable before and 1 hour after an ACTH stimulation test. For this reason, the mean value of DHEA-S was calculated for those moments.[18]

The data were analyzed with Statistical Analysis System (SAS 9.1, SAS Institute, Cary. NC, USA) software and were examined for the normality of the residuals (Cramér-von-Mises). For the multiple comparisons of mean DHEA-S between groups, the Tukey test (significance level = 0.05) was used.

III. Result

Concentrations of basal and post-ACTH cortisol levels of healthy dogs showed mean values (± standard deviation) of 12.32 ± 2.93 ng/mL (range, 8.4–15.2 ng/mL) and 114.19 ± 27.14 ng/mL (range, 81.1–148 ng/mL), respectively. Group B mean levels of basal and post-ACTH serum cortisol were 54.49 ng/mL ± 36.59 (range, 8.9–111 ng/mL) and 278.4 ng/mL ± 70.7 (range, 221.7–450 ng/mL), respectively. Finally, group C mean value of post-ACTH serum cortisol was 69.69 ± 38.14 ng/mL (range, 23.2–141.6 ng/mL) (Table 1).

Table 1. Pre-and post-ACTH cortisol levels of groups A (healthy dogs), B (dogs diagnosed with HAC), and C (dogs with HAC treated with trilostane)

	Group A	Group B	Group C
Basal cortisol	12.32 ng/mL ± 2.93	54.49 ng/mL ± 36.59	----
Post-ACTH cortisol	114.19 ng/mL ± 27.14	278.4 ng/mL ± 70.7	69.69 ng/mL ± 38.14

Serum DHEA-S levels (mean ± standard deviation) of group A (0.026 ± 0.031 µg/mL) did not differ from dogs in group B (0.030 ± 0.026 µg/mL) (p=0.9714). Serum concentrations of DHEA-S of dogs in group C (0.086 ± 0.013 µg/mL) were significantly higher than dogs in groups A (p = 0.0063) and B (p = 0.0037) (Figure 1).

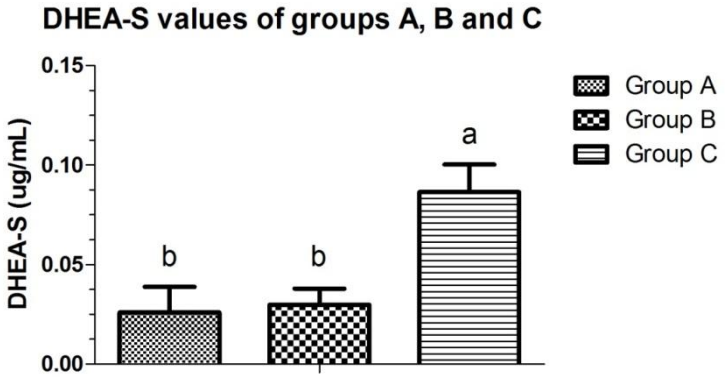

Figure 1- DHEA-S values of groups A (healthy dogs), B (dogs diagnosed with HAC) and C (dogs with HAC treated with trilostane). Different letters indicate a significant difference ($p < 0.05$, Tukey test). The horizontal bars represent the standard deviation.

IV. Discussion

The mean values of post-ACTH serum cortisol of groups A and B confirmed the reference values described in the literature for healthy dogs and dogs diagnosed with HAC, respectively.[12] Group B samples were collected from dogs diagnosed with HAC confirmed by cortisol measurement after the ACTH stimulation test and then classified as ACTH-dependent HAC through abdominal ultrasound and evaluation of the adrenal glands, which evidenced apparent symmetric bilateral hyperplasia. The adrenal gland assessment as an efficient technique to differentiate ACTH-dependent HAC from the independent form.[15]

Group C included dogs that had controlled clinical signs of HAC and were treated with trilostane. In these dogs, the ACTH stimulation test was performed every 2 weeks as the trilostane dose was rechecked. When the cortisol levels and clinical signs stabilized, hormonal monitoring was required less frequently, hence frequency of hormonal monitoring was decreased from once every 2 weeks to once every month or 3 months, or twice per year. Only the post-ACTH cortisol dosage was measured to reduce the costs to the owners. The clinical evaluation and one post-ACTH cortisol dosage can reliably be used to monitor patients treated with trilostane, making basal cortisol evaluation unnecessary.[3]

Monitoring of group C patients was performed1 and only clinically stable dogs were included in the study. All dogs presented improvement of clinical signs secondary to the disease, but only five dogs (5/9) had cortisol values within the normal reference parameters (post-ACTH, 20–50 ng/mL). The other four animals presented remission of clinical signs such as polyuria, polydipsia, polyphagia, hyperlipidemia, and skin problems, even with cortisol values higher than recommended for animals under treatment. Therefore, adjusting the trilostane dose for these patients was unnecessary.

Previous studies have evaluated DHEA-S concentrations in humans[6] and observed that DHEA-S concentrations diminished after tumor resection in human patients diagnosed with Cushing's syndrome and treated with transsphenoidalhypophysectomy, but then increased more rapidly than cortisol levels in patients with recurrence. Even though we have not confirmed pituitary tumors as the true cause of HAC, it was expected that dogs with HAC had greater DHEA-S concentrations than healthy dogs.[9]In this context, we demonstrated that dogs with HAC did not present higher DHEA-S levels than healthy dogs. These results are contradictory to those described previouslyby,[10] who evaluated sexual hormones in dogs with spontaneous hypercortisolism and observed increased DHEA-S levels compared to the normal reference values.

Authors in another study evaluated 15 human patients with spontaneous hypercortisolism through measurement of cortisol, DHEA, DHEA-S, and plasma levels of androstenedione to better understand the effect of the ACTH stimulation test on androgens. They found that ACTH does not control the secretion of androgens, as significant increases in these hormones compared to cortisol were not observed. This suggests that unknown

mechanisms can also control the production of sexual hormones and that excess ACTH is not a determinant for their constant increase.[7] ACTH was not the only stimulus for the production of sexual hormones, but that other hormones, such estrogen, prolactin, growth hormone, and gonadotropins, were involved in the control of androgens[14]. This may explain our findings, because dogs presenting with excessive cortisol levels secondary to high ACTH do not express a significant increase in DHEA-S levels when compared to healthy dogs, suggesting that other mechanisms that produce sexual hormones should be involved.

Interestingly, we observed greater DHEA-S concentrations in dogs treated with trilostane than in healthy dogs or dogs diagnosed with HAC that received no treatment. Trilostane is a reversible and competitive inhibitor of 3β-HSD, which is responsible for the conversion of DHEA into androstenedione in the synthesis of sexual hormones. Therefore, an increase in the DHEA-S concentration in dogs treated with trilostane can indicate that the treatment has an inhibitory effect on the production of sexual hormones, since DHEA-S is dissociated into DHEA prior to its conversion into androgens and estrogens through the sulfotransferase enzyme.[13,16,17]

Another possible reason that can explain the increase in DHEA-S concentrations in dogs treated with trilostane is the body's response to overcoming this pharmacologic inhibition. A block of androstenedione production (generated by trilostane) stimulates increased synthesis of DHEA to compensate for the secretory reduction, and increase the DHEA-S level in the blood. This argument can also be explained by the increase of ACTH secretion in dogs treated with trilostane.[19] ACTH is not the only agent responsible for the increase in the production of sexual hormones; however, some authors proved that levels of sexual hormones were significantly higher in dogs with ACTH-dependent HAC treated with trilostane than in dogs before starting treatment.[20,19]

We cannot infer the effect of DHEA-S in dogs with HAC treated with trilostane. Our two groups did not include the same patients at two different times, hence we cannot verify the secretion dynamics of this hormone. For this reason, in this study we cannot suggest that DHEA-S effectively increases due to trilostane treatment. However, in a previous study the DHEA concentration in dogs treated with trilostane and monitored for 7 weeks was significantly higher than that at the time of diagnosis, confirming the inhibitory effect of this drug on the 3β-HSD enzymatic system.[19] This was confirmed by our study, as DHEA-S concentrations were higher in dogs treated with trilostane than in untreated dogs with HAC.

In accordance with our results, we affirm that DHEA-S is not considerably increased in dogs with ACTH-dependent HAC, but is present in high concentrations in dogs treated with trilostane. Thus, it is a weak marker for the diagnosis of HAC in dogs, but could have a clinical implication for monitoring dogs treated with trilostane since its increase may be inversely proportional to the decrease of cortisol, for this we need other studies. No studies of healthy dogs treated with trilostane to confirm its enzymatic inhibition of DHEA-S have been published, which would help confirm these effects.

V. Conclusion

Dogs diagnosed with spontaneous hypercortisolism under treatment with trilostane had higher serum DHEA-S concentrations than dogs with untreated HAC or healthy dogs, possibly owing to the inhibitory effect of the drug on the 3β-HSD enzyme in the production of sexual hormones. The evaluation of DHEA-S did not prove promising to diagnose HAC and monitoring in dogs with HAC treated with trilostane could be better studied by correlating their values with cortisol values.

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