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Comparison of 2 Doses for ACTH Stimulation Testing in Dogs Suspected of or Treated for Hyperadrenocorticism

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Background: Lowering the cosyntropin dose needed for ACTH stimulation would make the test more economical.

Objectives: To compare the cortisol response to 1 and 5 μg/kg cosyntropin IV in dogs being screened for hyperadrenocorticism (HAC) and in dogs receiving trilostane or mitotane for pituitary-dependent HAC.

Animals: Healthy dogs (n = 10); client-owned dogs suspected of having HAC (n = 39) or being treated for pituitary-dependent HAC with mitotane (n = 12) or trilostane (n = 15).

Procedures: In this prospective study, healthy dogs had consecutive ACTH stimulation tests to ensure 2 tests could be performed in sequence. For the first test, cosyntropin (1 μg/kg IV) was administered; the second test was initiated 4 hours after the start of the first (5 μg/kg cosyntropin IV). Dogs suspected of having HAC or being treated with mitotane were tested as the healthy dogs. Dogs receiving trilostane treatment were tested on consecutive days at the same time post pill using the low dose on day 1.

Results: In dogs being treated with mitotane or trilostane, the 2 doses were pharmacodynamically equivalent (90% confidence interval, 85.1–108.2%; $P = 0.014$). However, in dogs suspected of having HAC, the doses were not pharmacodynamically equivalent (90% confidence interval, 73.2–92.8%; $P = 0.37$); furthermore, in 23% of the dogs, clinical interpretation of test results was different between the doses.

Conclusions and Clinical Relevance: For dogs suspected of having HAC, 5 μg/kg cosyntropin IV is still recommended for ACTH stimulation testing. For dogs receiving mitotane or trilostane treatment, a dose of 1 μg/kg cosyntropin IV can be used.

Key words: Adrenal; Cosyntropin; Mitotane; Trilostane.

Spontaneous hyperadrenocorticism (HAC) is a frequently diagnosed endocrinopathy in dogs. The disease can be devastating to both the dog and the owner(s). Since HAC has a myriad of clinical signs and is common in clinical practice, veterinarians frequently screen for the disease. The ACTH stimulation test is one of the main screening tests used to diagnose HAC.[1]

Abbreviations:

HAC	hyperadrenocorticism
PDH	pituitary-dependent hyperadrenocorticism

The mainstays of medical treatment for HAC are mitotane and trilostane. For both medications, dosages must be titrated to effect and the dog supervised closely on an ongoing basis. Without appropriate monitoring, either medication can fail to control the disease, cause adverse effects including hypoadrenocorticism, which can be fatal, or both.[2–7] Dose assessment is best performed by evaluating clinical response in conjunction with an ACTH stimulation test. The preferred form of ACTH, cosyntropin, is expensive, and, as a result, the testing required for dosage adjustment and appropriate therapeutic monitoring can be cost-prohibitive. Although compounded ACTH is obtainable, timing of response can be variable,[8] and quality control varies between sources. Use of a lower cosyntropin dose could greatly decrease the cost of an ACTH stimulation test and allow more owners to appropriately monitor HAC treatment in their pets.

The goal of an ACTH stimulation test is to maximally stimulate adrenocortical cortisol secretion. The currently accepted cosyntropin dose is 5 μg/kg IV or IM with sampling at 0 and 60 minutes.[9,10] In healthy dogs, 1 μg/kg provides the same maximal adrenocortical stimulation as 5 μg/kg and 250 μg/dog IV.[9,11,12] However, the lower 1 μg/kg dose has not been evaluated in dogs suspected to have spontaneously occurring HAC or in dogs receiving mitotane or trilostane treatment.

Therefore, our objectives were: (1) To compare the serum cortisol response when 1 and 5 μg/kg

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cosyntropin IV was administered to dogs being screened for HAC. (2) To compare the serum cortisol response when 1 and 5 μg/kg cosyntropin IV was administered to dogs receiving trilostane or mitotane for treatment of pituitary-dependent hyperadrenocorticism (PDH). Our hypothesis was that 1 μg/kg cosyntropin IV can be used to screen dogs for HAC and monitor treatment in dogs receiving mitotane or trilostane for treatment of HAC.

Materials and Methods

All studies were approved by the Institutional Animal Care and Use Committee of Auburn University.

Phase 1: Healthy Dogs

In order to ensure that 2 ACTH stimulation tests could be done in a single day without altering the results of the second test, 10 clinically healthy dogs owned by students, staff, or faculty of the Auburn University Veterinary Teaching Hospital were used. All dogs were enrolled with the informed consent of their owners. Dogs were deemed clinically healthy on the basis of history, physical examination, and results of a CBC, serum biochemical analysis, urinalysis, and occult heartworm test. Dogs that were <1 year of age, unneutered, <3 kg (6.6 lbs) or with known or suspected adrenal gland disease were excluded. Additional exclusion criteria included receiving oral or topical glucocorticoid treatment in the previous month, injectable glucocorticoid treatment in the previous 2 months, ketoconazole or progestin treatment in the previous month, or etomidate or anesthesia in the previous 7 days.

Each dog underwent 2 ACTH stimulation tests in a single day. For the first test, a dose of 1 μg/kg cosyntropin[a] was administered IV (low-dose test). Blood samples were obtained just before injection (hour 0) and 60 minutes post injection (hour 1). Three hours after collection of the postsample (hour 4), a third blood sample was obtained and a second dose of cosyntropin (5 μg/kg IV) was administered (high-dose test). The last blood sample was drawn 1 hour later (hour 5).

Phase 2: Dogs Suspected of Having HAC or Being Treated for PDH

Client-owned dogs suspected of having HAC or that were being treated for PDH with either mitotane or trilostane were enrolled. Exclusion criteria with regard to age, weight, and medication history were the same as for the healthy dogs. Dogs with adrenal tumors were also excluded.

Participants were enrolled at 2 university veterinary teaching hospitals, 1 specialty private practice, and 3 general practices. For those being treated for PDH, the presence of HAC was suspected on the basis of consistent history, physical examination findings, and routine laboratory testing. A diagnosis of PDH had been confirmed using the results of a previous ACTH stimulation test not done as part of this study, a low-dose dexamethasone suppression test, or both. Differentiation between PDH and an adrenal tumor was determined via a high-dose dexamethasone suppression test, measurement of endogenous ACTH concentration, or abdominal ultrasound. For those receiving treatment for PDH, the dogs could have been treated for any length of time before enrollment. For dogs receiving mitotane, testing could have been in the loading or maintenance phase.

For dogs suspected of having HAC or those receiving mitotane, ACTH stimulation tests were performed as described for the healthy dogs. As ACTH stimulation tests for dogs receiving trilostane must be initiated at a specific time post pill, both tests could not be performed in a single day. Accordingly, a test using a 1 μg/kg dose of cosyntropin IV was performed on day 1, and the second test (5 μg/kg cosyntropin IV) was performed the following day.[13] All tests were initiated 4–6 hours post pill on day 1; for a particular dog, the second test was started at the same time post pill as on day 1. For both mitotane and trilostane, dogs with 60-minute post-ACTH cortisol concentrations <1.1 μg/dL (30 nmol/L) were considered to be excessively treated. An ACTH-stimulated cortisol concentration of 1.1–5.4 μg/dL (30–150 nmol/L) was considered to be in the ideal therapeutic range. An ACTH-stimulated cortisol concentration >5.4–9.0 μg/dL (>150–250 nmol/L) was considered acceptable if clinical signs were controlled. Finally, dogs with ACTH-stimulated cortisol concentrations >9.0 μg/dL (>250 nmol/L) were considered to have inadequate control.[2]

Preparation of Cosyntropin

Cosyntropin was supplied as 250 μg of lyophilized powder in 2-mL vials. Each vial was reconstituted with 1.0 mL sterile saline solution, in accordance with the manufacturer's directions. Unused cosyntropin remaining after reconstitution was frozen in 50-μg aliquots in plastic syringes until use or for no longer than 4 months.[14] To administer the lower dose to small dogs, the cosyntropin was diluted to a concentration of 10 μg/mL, and stored frozen at −20°C in plastic syringes in 5-, 10-, and 20-μg aliquots.[15] To achieve this dilution, the original 1.0 mL (250 μg) of reconstituted cosyntropin was added to 24.0 mL sterile saline to achieve a final volume of 25.0 mL. No cosyntropin was thawed and refrozen.

Assay Procedures

All samples were placed into plain collection tubes and centrifuged after clotting within 1 hour of collection. The serum was separated and stored at −20°C until analysis. Serum cortisol concentrations were measured using a previously validated radioimmunoassay.[b,11] Sensitivity of the assay was 0.5 μg/dL. For statistical purposes, values below the sensitivity of the assay were recorded as 0.3 μg/dL. The intra- and interassay coefficients of variation are 5.1 and 10.3%, respectively.[11] All samples from healthy dogs were assayed in a single batch. For dogs suspected of having HAC or being treated for PDH, samples were assayed in multiple batches; however, samples from each dog were run in a single batch when possible. All samples were assayed in duplicate.

Statistical Analyses

Statistical analysis was performed using commercial software packages.[c,d,e] For all tests, significance was set at the $P < 0.05$ level. The Shapiro–Wilk test was used to evaluate normality of cortisol concentration, age, and weight data, and all were determined to be not normally distributed. Thus, data are presented as median (range).

Pharmacologic equivalence of the ACTH doses was tested by determining if the 90% confidence interval (CI) for the ratio of the mean ACTH-stimulated cortisol concentration in response to each dose was fully contained within the equivalence region of 80 and 125% for the ratio. Age and weight of the dogs treated with either medication were compared using a Mann–Whitney rank sum test. Age and weight of the treated dogs and dogs suspected of having HAC were compared using an ANOVA on ranks.

Results

Phase 1

Population. Median age of the healthy dogs was 4.5 years (1.0–7.1 years) and median weight was

14.1 kg (4.0–30.2 kg). There were 6 female and 4 male dogs; all were spayed or neutered. Breeds represented included miniature Dachshund (n = 3) and one each of Labrador retriever, Boston Terrier, and Vizsla. Four dogs were mixed breeds.

Cortisol Concentrations. Administration of both doses of ACTH caused an increase in cortisol concentration (Table 1). The ACTH-stimulated cortisol concentrations in response to both doses were equivalent (90% confidence interval, 86.6–99.3%; $P = 0.0015$).

Phase 2—Dogs Suspected of Having HAC or Being Treated for PDH

Population. Thirty-nine dogs suspected of having HAC were included. Twenty dogs were spayed females and 19 were neutered males. Breeds included were Labrador retriever (n = 4), miniature Schnauzer (n = 3), Maltese (n = 3), English bulldog (n = 3), Shih Tzu (n = 2), and one each of Australian shepherd, Bassett hound, Cocker spaniel, Beagle, Bichon Frise, Border collie, Chihuahua, Chow Chow, Dachshund, Doberman Pinscher, German shepherd dog, Lhasa Apso, Mastiff, Pomeranian, Scottish Terrier, Sealyham Terrier, toy poodle, Vizsla, West Highland white Terrier, and Yorkshire Terrier. Two dogs were of mixed breed and 2 breeds were unrecorded. Median weight was 13.9 kg (3.6–55.6 kg) and median age was 10.0 years (4.0–16.0 years).

Twelve dogs being treated with mitotane for PDH were included. Seven dogs were spayed females and 5 were neutered males. Breeds included were Shih Tzu (n = 3) and one each of American bulldog, Chihuahua, Parson Russell Terrier, miniature Dachshund, Labrador retriever, Pomeranian, Staffordshire Terrier, and Yorkshire Terrier. One dog was of mixed breed. Median weight was 9.0 kg (3.7–34.8 kg) and median age was 12.0 years (7.0–13.0 years). Fifteen dogs being treated with trilostane for PDH were included. Nine dogs were spayed females and 6 were neutered males. One each of American Cocker spaniel, Beagle, Chihuahua, English bulldog, Parson Russell Terrier, Lhasa Apso, miniature Dachshund, miniature Pinscher, and Yorkshire Terrier were included. Six dogs were of mixed breed. Median weight was 15.5 kg (2.6–26.4 kg) and median age was 12.0 years (5.0–14.0 years).

Age and weight were not significantly different among dogs suspected of HAC or being treated for PDH ($P = 0.51$ and 0.274, respectively). Age and weight were not significantly different between dogs treated with mitotane or trilostane ($P = 0.66$ and 0.71, respectively).

Cortisol Concentrations. For the dogs suspected of having HAC, the ACTH-stimulated cortisol concentrations in response to both doses of ACTH were not equivalent (90% confidence interval, 73.2–92.8%; $P = 0.37$; Table 1). In addition, the clinical interpretation of the test results was different between the 2 doses in 9 dogs (23% discordance; Fig 1). Three of the 9 dogs had an ACTH-stimulated cortisol concentration that was lower after administration of the 5 μg/kg dose than after administration of a 1 μg/kg dose. For the other 6 dogs, the ACTH-stimulated cortisol concentration was higher after administration of the 5 μg/kg dose, and in 4 of these 6, the test results were within the reference range on the low-dose test and were consistent with a diagnosis of HAC on the high-dose test.

In the dogs being treated for PDH, the ACTH-stimulated cortisol concentrations in response to both doses were equivalent (90% confidence interval, 85.1–108.2%; $P = 0.014$; Table 1). The clinical interpretation of the test results was different between the 2 doses in 4 dogs (33%) receiving mitotane treatment. In 2 dogs, the interpretation of the ACTH-stimulated cortisol concentration after the low dose would be that the control was acceptable (cortisol concentrations 5.9 and 6.1 μg/dL); after the high dose, interpretation would be that the control was ideal (concentrations 1.4 and 4.4 μg/dL). In the third dog, interpretation after the low and high dose would be that the control was ideal and acceptable, respectively (cortisol concentrations 2.5 and 6.7 μg/dL,

Table 1. Basal and ACTH-stimulated cortisol concentrations median (range) from 2 ACTH stimulation tests in healthy dogs, in dogs suspected of having hyperadrenocorticism (HAC) and in dogs being treated for pituitary-dependent hyperadrenocorticism (PDH) with trilostane or mitotane.

Cosyntropin dose	1 μg/kg		5 μg/kg	
	Baseline cortisol concentration (μg/dL)	ACTH-stimulated cortisol concentration (μg/dL)	Baseline cortisol concentration (μg/dL)	ACTH-stimulated cortisol concentration (μg/dL)
Healthy dogs (n = 10)	1.7 (0.9–4.7)	10.4 (7.4–16.9)	1.0 (0.7–2.5)	11.1 (7.7–19.6)
Dogs suspected of having HAC (n = 39)	3.6 (0.3–22.9)	17.5 (5.1–58.4)	2.2 (0.4–8.9)	18.8 (6.4–83.0)
Dogs being treated for PDH with trilostane (n = 15)	2.1 (0.3–7.1)	2.5 (0.4–30.4)	1.9 (0.5–9.9)	2.3 (0.7–27.6)
Dogs being treated for PDH with mitotane (n = 12)	4.3 (0.8–7.1)	6.0 (0.6–27.5)	3.7 (1.1–10.9)	5.8 (1.4–31.5)

For healthy dogs, dogs suspected of having HAC and dogs being treated with mitotane for PDH, a dose of 1 μg/kg cosyntropin IV was administered first followed by a dose of 5 μg/kg IV 3 hours after conclusion of the first ACTH stimulation test. For dogs being treated with trilostane for PDH, a dose of 1 μg/kg IV was administered 4–6 hours post pill on day 1. The second test was performed at the same time post pill on the following day using a dose of 5 μg/kg IV. Data are presented as median (range). In dogs suspected of having HAC, the doses were not equivalent. In healthy dogs and dogs being treated for PDH, the doses were found to be equivalent.

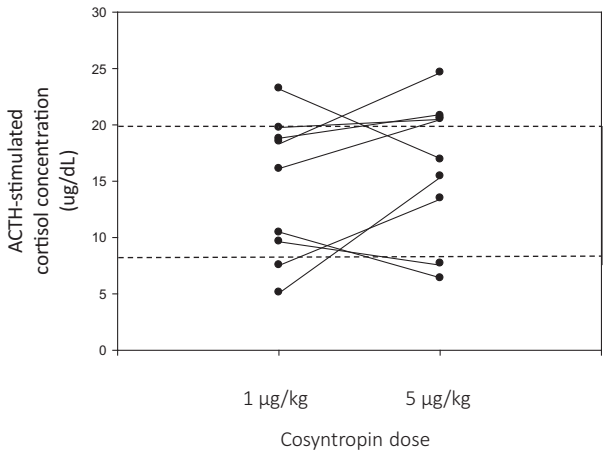

Fig 1. ACTH-stimulated cortisol concentrations in 9 dogs suspected of hyperadrenocorticism for which interpretation of the 2 ACTH stimulation tests differed. The reference range for ACTH-stimulated cortisol concentration is 8.0–20.0 μg/dL; the lower and upper limits are shown by the dotted lines.

respectively). In the fourth dog, interpretation after the low and high dose would be that the control was excessive and ideal, respectively (concentrations 0.6 and 1.4 μg/dL, respectively). The clinical interpretation of the test results was different between the 2 doses in 1 dog (7%) receiving trilostane treatment. Interpretation after the low and high dose would be that the control was acceptable and inadequate, respectively (concentrations 8.9 and 11.2 μg/dL, respectively).

Discussion

In this study, the 2 cosyntropin doses were not pharmacodynamically equivalent in dogs suspected of having HAC, and the clinical test interpretation would have been different in 23% of the dogs. In dogs being treated for PDH with mitotane or trilostane, the 2 cosyntropin doses were judged to be pharmacodynamically equivalent; test interpretation was different in 19% overall (4 dogs treated with mitotane and 1 dog treated with trilostane). The clinical discordance was more worrisome with respect to the type of assessment that would be made in the dogs suspected of having HAC than in those being treated for PDH.

In the first part of the study using healthy dogs, equivalence testing found that poststimulation cortisol concentrations for a 1 μg/kg dose were equivalent to poststimulation cortisol concentrations for a 5 μg/kg dose. Thus, for dogs suspected of having HAC or receiving mitotane for treatment of PDH, the dogs were able to be tested in a single day. The ACTH stimulation tests for dogs receiving trilostane must be initiated at a specific time post pill. A change of even 2 hours can significantly alter the ACTH-stimulated cortisol concentration,[16] so both tests could not be performed in a single day on dogs receiving trilostane. Administration of cosyntropin was previously documented to not affect the results of an ACTH stimulation test performed 24 hours later.[13] Thus, for dogs receiving trilostane, tests were administered on consecutive days, starting at the same time post pill.

In previous studies of healthy dogs, a 1 μg/kg IV cosyntropin dose provided maximal adrenal stimulation.[9,11,12] In addition, in 7 dogs with HAC, ACTH-stimulated cortisol concentrations were not significantly different when comparing cosyntropin doses of 5 μg/kg and 250 μg/dog IV.[9] In the current study we included dogs suspected of having HAC, not only those proven to have HAC, to simulate the population of dogs tested in practice.

Test interpretation was different in 9 of the 39 dogs suspected of having HAC. In the previous study including 7 dogs with HAC, test interpretation did not change in any dog.[9] In 4 dogs in the current study, the result of the low-dose test was within the reference range while those of the high-dose test were consistent with a diagnosis of HAC, a crucial difference. Changes in test interpretation in the dogs being treated for PDH were less worrisome. In 3 of the 5 dogs with discrepant results, results of one of the tests showed ideal control and the other test showed acceptable control. Thus, although technically different results, clinically they were similar. Clinical signs must always be used as part of the clinical evaluation and to judge whether a dose of mitotane or trilostane should be altered, further minimizing the effect of the discrepant results.

A few important considerations exist if using the lower dose of cosyntropin. First, timing of the ACTH-stimulated cortisol concentration is crucial. When using a dose of 5 μg/kg IV, although a sample at 60 minutes post ACTH is recommended to make the test as short as possible, samples can be taken up to 90 minutes post ACTH administration allowing flexibility. When a dose of 1 μg/kg is given, the duration of peak ACTH-stimulated cortisol concentrations is much shorter, at least in healthy dogs, and, therefore, the 60-minute postsample time should be strictly used.[9,12] Second, in small dogs in the current study, the cosyntropin was diluted in order to achieve accurate dosing. Diluted Cortrosyn® solutions remain fully stable (in concentrations as low as 0.5 μg/mL) for at least 4 months when refrigerated in plastic containers.[15] At a higher concentration, that is, 250 μg/mL, the product is stable frozen at −20°C for up to 6 months.[14] Stability of other cosyntropin products during storage is unknown. Third, only the intravenous route of administration can be recommended for use of the low dose, as intramuscular administration was not evaluated.

In conclusion, when testing dogs for HAC, a dose of 5 μg/kg cosyntropin is still recommended. When monitoring treatment with mitotane or trilostane, a lower dose of 1 μg/kg can be used and results interpreted in conjunction with assessment of control of clinical signs. The timing of the post-ACTH sample when using a lower dose of cosyntropin is crucial and the ACTH must be given IV. Cortrosyn® is recommended as the stability of the product during storage has been proven.

Footnotes

[a] Cortrosyn®, Amphastar Pharmaceuticals Inc., Rancho Cucamonga, CA
[b] Coat-A-Count cortisol assay, Siemens Medical Solution Diagnostics, Los Angeles, CA
[c] Equivtest 2.0, Statistical Solutions, Boston, MA
[d] SigmaPlot 12.0, Systat Software, Inc, Chicago, IL
[e] NCSS 10, NCSS, LLC, Kaysville, UT

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Conflict of Interest Declaration: Authors declare no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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