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Journal

Journal of Veterinary Internal Medicine, 31(3)

ISSN

0891-6640

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Publication Date

2017-05-01

DOI

10.1111/jvim.14708

Peer reviewed

Journal of Veterinary Internal Medicine
Open Access
ACVIM American College of Veterinary Internal Medicine

Standard Article

J Vet Intern Med 2017;31:730–733

Effect of Intravenous or Perivascular Injection of Synthetic Adrenocorticotropic Hormone on Stimulation Test Results in Dogs

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Background: Standard protocols for adrenocorticotropic hormone (ACTH) stimulation testing (ACTHst) often involve intravenous (IV) injection of corticotropin. ACTH might be unintentionally injected into the perivascular (PV) space.

Objective: To compare stimulation test results after IV and PV injections of ACTH.

Animals: Twenty privately owned dogs were studied: 10 healthy and 10 with trilostane-treated naturally occurring hyperadrenocorticism (HAC).

Methods: Prospective study. Each of 20 dogs underwent 2 ACTHst not <4 nor more than 14 days apart. Five healthy and 5 HAC dogs had an IV ACTHst first and PV second; 5 healthy and 5 HAC dogs had a PV ACTHst first and IV second. Blood samples for measurement of serum cortisol concentration were collected before and 1 hour after ACTH administration.

Results: No significant difference in results was demonstrated when comparing serum cortisol concentrations after IV and PV ACTH administration in all 20 dogs (median μg/dL; interval μg/dL: 8.2; 1.4–17.4 versus 7.8; 0.9–16.9; $P = .23$). No significant difference in results was demonstrated when comparing serum cortisol concentrations after IV and PV ACTH administration in the 10 healthy dogs (median μg/dL; interval μg/dL: 10.9; 7.3–17.4 versus 10.6; 7.1–16.9; $P = .54$) or in the 10 HAC dogs (median μg/dL; interval μg/dL: 6.3; 1.4–8.6 versus 5.2; 0.9–8.7; $P = .061$).

Conclusions and Clinical Importance: Perivascular administration of ACTH does not significantly alter stimulation test results in healthy dogs or in dogs with HAC undergoing therapy with trilostane.

Key words: Hyperadrenocorticism; Endocrinology; Adrenal; Pituitary; Trilostane.

Serum or plasma cortisol concentrations in blood samples obtained before and after adrenocorticotropic hormone (ACTH) administration are commonly used for confirming a diagnosis of hyperadrenocorticism (HAC) or hypoadrenocorticism in dogs.[1–5] Results of the ACTH stimulation test (ACTHst) are also used to aid in determining optimal dose and frequency of medications used in the short and long-term management of dogs with HAC.[4,6–8] Alpha 1–24 synthetic corticotropins, potent synthetic subunits of ACTH, are commonly used in veterinary medicine.[9,10] Several forms of synthetic ACTH are commercially available, one is a sterile lyophilized powder in vials containing 0.25 mg of cosyntropin, which is recommended for intravenous (IV) or intramuscular administration after it is reconstituted[a] and another is a liquid synthetic ACTH product available for IV use only.[b] The description of the amino acid sequence of these 2 products is identical, and their actions are similar.[11]

Abbreviations:

ACTH	adrenocorticotropic hormone
ACTHst	ACTH stimulation test
HAC	hyperadrenocorticism
IV	intravenous
PV	perivascular

Cost of ACTH formulations in the USA has escalated dramatically over the past several decades. Adding the cost of ACTH to fees for obtaining the necessary blood samples plus charges for assaying each sample for cortisol can be considerable, causing some owners to decline having the ACTHst performed. This concern has led to the development of strategies to reduce the cost of ACTHst. One approach was to dose ACTH based on body weight as opposed to the one-large-dose-fits-all, previously used.[2,12,13] The low-dose ACTHst provides consistent results at decreased cost.[2,12,13] Other approaches have been designed to attempt to avoid the use of ACTHst altogether. Several groups assessed sensitivity and specificity of a variety indices for confirming a diagnosis of Addison's disease without use of ACTHst.[5,14,15] Other groups have evaluated non-ACTHst indices as aids in trilostane management of dogs with HAC.[16–19] Cost of ACTH is consistently mentioned as an indication for performing such studies.

Despite alternative approaches to diagnosis and monitoring that might avoid its use, the ACTHst remains a "test of choice" and is the test against which other diagnostic or monitoring protocols are often

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Animal evaluation and data collection took place at the Animal Specialty and Emergency Center in Los Angeles, CA. Sample analysis was performed by the UC Davis School of Veterinary Medicine clinical laboratory services.

This article has not been presented at any meeting.

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Submitted December 23, 2016; Revised February 22, 2017; Accepted March 2, 2017.

DOI: 10.1111/jvim.14708

judged.[12–19] Many veterinarians utilize the ACTHst whenever an owner allows, because results of this test continue to provide seemingly straightforward criteria for making a correct decision, whether regarding diagnosis or assessing response to therapy. Occasionally, however, factors such as method of restraint, venipuncture technique, patient body condition, or patient temperament might result in extravasation of some or of all ACTH into the perivascular (PV) space during intended IV administration. The effect of PV injection of ACTH on the ACTHst is unknown.

The purpose of this study was to evaluate ACTHst results when an entire dose of ACTH is administered IV into the cephalic or saphenous vein, as directed on the drug insert, compared with results obtained after an entire dose of ACTH is injected at the same site but into the PV space in a group of healthy dogs and in a group of dogs being treated with trilostane for naturally occurring HAC. This hypothesis was that ACTHst results would be similar regardless of administration site.

Materials and Methods

Twenty privately owned pet dogs were studied between July 2015 and July 2016. Each dog was a patient or an employee-owned pet of the Animal Specialty and Emergency Center in Los Angeles, CA. Informed consent was obtained from each owner before enrollment. No dog had received glucocorticoid medication in the 4 months preceding or during the study. No dog could be given any new medication nor could any change in dose of trilostane be made during the time period between the 2 ACTHst. Ten healthy dogs and 10 dogs previously diagnosed as having naturally occurring HAC were included.

The 10 healthy dogs included 6 castrated males and 4 spayed females. Breeds represented included Cocker Spaniel (1), Labrador retriever (1), Boxer (1), and 7 mixed breed dogs. The median age was 6.5 years with a range of 1–14 years. Median body weight of this population was 19.5 kg with a range of 6–43 kg. Owners of each healthy dog believed their dog was well, each was considered healthy on physical examination, and each had within-reference-limit-results on CBC, serum biochemistry profile, and urinalysis. The group of 10 dogs with HAC included 4 castrated males and 6 spayed females. Breeds represented included Akita (1), Alaskan Malamute (1), Bichon Frise (1), Boston terrier (1), Chihuahua (1), Jack Russell Terrier (1), Labradoodle (1), Miniature Poodle (1), Rhodesian Ridgeback (1), and mixed breed (1). The median age was 9.5 years with a range of 8–13 years. Median body weight of this population was 17.4 kg with a range of 4.2–46 kg. Both the healthy and the HAC dogs included 2 dogs each weighing 5–10, 10–15, 15–20, 20–25 and >25 kg. At the time of diagnosis, dogs with HAC must have had clinical signs, physical examinations, and routine biochemical findings typical for the condition. Each dog with HAC must have had an ACTHst result or low-dose dexamethasone suppression test result consistent with the diagnosis. Each of the 10 HAC dogs had been treated with trilostane[c] (given with food) twice daily for at least 1 month, and each was stable before inclusion in this study, without signs of illness aside from those commonly associated with HAC. Median trilostane dosage was 0.85 mg/kg, PO q12h, with a range of 0.4–2.4 mg/kg. Clinical status and body weight did not change for any of the 20 dogs during the interval between first and second test.

Each dog underwent 2 ACTHst, one with ACTH administered IV and the other with ACTH given PV. All tests were conducted using 5 μg/kg synthetic ACTH[b], with volumes of ACTH administered ranging from 0.1 to 1 mL. The second ACTHst was conducted a minimum of 4 to a maximum of 14 days after the first. All ACTHst in the healthy dogs were started between noon and 3 PM and in each HAC dog 4–6 hours after trilostane administration[c]. In each dog, 1 vein was used for all testing. Five healthy dogs and 5 HAC dogs were each randomly assigned to be tested first with the ACTH given IV: groups 1 and 3, respectively. Five healthy and 5 HAC dogs were each randomly assigned to be tested first with the ACTH administered into the PV space directly over the vein: groups 2 and 4, respectively. Blood samples (2–3 mL) were taken immediately before and 1 hour after ACTH administration, from the same vein (saphenous or cephalic) into which or next to which ACTH had been administered. Blood was collected into additive-free plastic tubes, allowed to clot, spun down, separated, and the serum frozen at −18°C until analysis. All serum samples were assayed together after all 20 dogs had completed both tests. The cortisol concentration in each serum sample was determined using a validated solid phase chemiluminescent immunoassay with an analytic sensitivity of 0.2 μg/dL (5.5 nmol/L).[e,7,17,21]

The study was originally powered for 20 dogs to find an effect size of 1.0 ± 1.5 μg/dL with $\alpha = 0.05$ and $ß = 0.20$. The exact Wilcoxon signed-rank test for paired data was used to evaluate the differences in serum cortisol concentrations before and after IV and PV administration. Analyses were performed conditional on disease and pre/post-treatment status, as well as only conditional on pre/post-treatment.[d] P-values < .05 were considered statistically significant.

Results

The distribution of basal serum cortisol concentrations before the first ACTHst in all 20 dogs (median 1.9 μg/dL; interval, 0.4–7.2 μg/dL) was not significantly different ($P = .59$) from the distribution of basal serum cortisol concentrations before the second test (median 2.4 μg/dL interval, 0.4–8.4 μg/dL; reference range: 0.5–6.0 μg/dL). These results are consistent with the first ACTHst having no residual effect on the second test result. Baseline serum cortisol concentrations before IV ACTH were merged, as were those before PV administration. The post-IV ACTH serum cortisol concentrations were merged as were the results post-PV.

The distribution of serum cortisol concentrations before IV ACTH administration in the healthy dogs (median 2.5 μg/dL; interval, 0.8–7.2 μg/dL) was not significantly different ($P = .63$) from the distribution before PV ACTH administration in the healthy dogs (median 2.0 μg/dL; interval, 0.7–8.4 μg/dL). The basal serum cortisol concentration before IV ACTH administration in the HAC dogs (median 1.9 μg/dL; interval, 0.4–5.3 μg/dL) was not significantly different ($P = .91$) from the cortisol concentration before PV ACTH administration in the HAC dogs (median 2.6 μg/dL; interval, 0.4–4.9 μg/dL).

The distribution of serum cortisol concentrations after IV ACTH administration in all 20 dogs (median 8.2 μg/dL; interval, 1.4–17.4 μg/dL; reference range 6–17 μg/dL) was not significantly different ($P = .23$) from the distribution obtained after PV ACTH administration (median 7.8 μg/dL; interval, 0.9–16.9 μg/dL). Adverse reactions were not seen in any dog during or

after any ACTHst. The distribution of serum cortisol concentrations after IV ACTH administration in the healthy dogs (median 10.9 μg/dL; interval, 7.3–17.4 μg/dL) was not significantly different ($P = .54$) from the distribution of serum cortisol concentrations after PV ACTH administration in the healthy dogs (median 10.6 μg/dL; interval, 7.1–16.9 μg/dL). The serum cortisol concentration after IV ACTH was higher in 4 of the 10 healthy dogs and higher after PV administration in 6. The difference in individual dogs between the post-ACTH concentrations after IV and PV administrations was <0.7 μg/dL in 8 of the 10 healthy dogs. In 1 healthy dog, the post-IV-ACTH serum cortisol concentration was 2.1 μg/dL higher than after PV: 11.5 and 9.4 μg/dL, respectively. In 1 healthy dog, the post-IV-ACTH serum cortisol concentration was 1.5 μg/dL lower than after PV: 12.6 and 14.1 μg/dL, respectively.

The distribution of serum cortisol concentrations after IV ACTH administration in the HAC dogs (median 6.3 μg/dL; interval, 1.4–8.6 μg/dL) was not significantly different ($P = .061$) from the distribution of serum cortisol concentrations after PV administration (median 5.2 μg/dL; interval, 0.9–8.7 μg/dL). Although the serum cortisol concentrations after IV ACTH were higher than after PV in 8 of the 10 HAC dogs, the difference between serum cortisol concentrations after IV versus after PV ACTH was <1.0 μg/dL in 5 of the 10 HAC dogs. In 4 HAC dogs, the post-IV-ACTH serum cortisol concentration was higher than after the PV ACTH by 1.2, 1.2, 1.6, and 4.2 μg/dL, respectively. In 1 HAC dog, the post-IV-ACTH serum cortisol concentration was 1.3 μg/dL lower than after PV ACTH.

Discussion

The ACTHst is frequently used for monitoring of HAC dogs undergoing treatment with trilostane. The dose of trilostane may subsequently be adjusted based on a combination of clinical signs and the more objective ACTHst results. Although both IV and intramuscular routes of ACTH administration are considered acceptable, subcutaneous administration of ACTH has never been evaluated so extravasation of an intended IV injection of ACTH during the course of an ACTHst typically results in aborting the test, or administering a repeat dose of ACTH, incurring additional cost. Data from all 20 dogs in this study support the reliability of results after PV administration of ACTH. No significant difference was found when comparing serum cortisol concentrations after IV and PV ACTH administration in normal or HAC dogs, suggesting that extravasation of ACTH is unlikely to significantly change results. An arbitrary goal during trilostane treatment is a post-ACTHst serum cortisol concentration between 1.5 and 9.1 μg/dL[c]. Paired serum cortisol concentrations from 1 HAC dog were both <1.5 μg/dL, 8 HAC dogs had both values between 1.5 and 9.1 μg/dL, and 1 dog had a post-IV ACTH serum cortisol concentration of 2.1 μg/dL and the post-PV result in that dog was 0.9 μg/dL. Thus, decisions regarding dose and frequency of trilostane administration would not have been different whether post-IV or post-PV results were employed in 9 of the 10 dogs with HAC in this study.

The difference in paired post-IV and post-PV ACTHst results in dogs with HAC was not statistically significant. However, there was a greater discrepancy between these values among HAC dogs than in healthy dogs. In the healthy dogs, the difference in post-ACTHst cortisol concentrations in 2/10 pairs was >0.7, whereas in the HAC dogs, the difference in post-ACTHst cortisol concentrations in 7 of 10 pairs was ≥0.7 μg/dL. Eight of 10 HAC dogs were found to have higher serum cortisol concentrations after the ACTH was given IV. It is possible that dogs with HAC are less capable of absorbing ACTH administered PV than the healthy dogs, which could explain these results. An alternative interpretation of these data from dogs with HAC could be based on their trilostane therapy. Trilostane effect on cortisol results may be quite different, either enhanced or diminished, if one of the paired ACTHst began as much as 1–2 hours earlier or later than the other. It seems unlikely that ACTHst results in healthy dogs would be affected by the time of testing, for example, starting the ACTHst at 12:30 PM for 1 test versus 2:15 PM for the other. However, varying the time of starting an ACTHst relative to the time of trilostane administration by as little as 2 hours has been demonstrated to significantly alter results.[20] Thus, results from the healthy dogs in this study likely better indicate the similarity in response to IV and PV ACTH. Even if testing was initiated at a specific time after trilostane administration, the systemic response to this drug may have changed in some dogs in the 4–14 days between tests.

If the variability found in cortisol results after PV versus IV testing in HAC dogs in this study is indeed related to timing of trilostane dose, then variability in cortisol results after PV versus IV administration of ACTH when screening dogs for HAC (not undergoing medical therapy) may be of less concern. However, we did not evaluate this issue nor did we study dogs with suspected hypoadrenocorticism.

It is also relevant to note that in this study, the complete dose of ACTH was given PV. This is in contrast to a more realistic scenario in veterinary practice. Extravasation, when it occurs, likely involves only a percentage of the administered ACTH. This might indicate that in most clinical scenarios, there will be even less effect on ACTHst results. However, this is theoretical, as this study did not evaluate such a condition. Given that a 1 μg/kg dose of ACTH has been shown to be pharmacodynamically equivalent to a 5 μg/kg dose in dogs with HAC being treated with mitotane or trilostane,[2] partial extravasation of the 5 μg/kg dose of ACTH may still cause maximal adrenocortical response. Formation of a hematoma with or after PV administration might influence effectiveness. No dog in this study developed a hematoma.

Results of this study confirmed this hypothesis and have shown that ACTHst results are not significantly different when ACTH is administered PV instead of IV in normal dogs and in dogs with HAC undergoing therapy with trilostane. Based on these findings, the inadvertent PV administration of ACTH is unlikely to

significantly affect results in a dog with HAC undergoing a routine stimulation test. Conducting a similar study in a larger cohort of dogs with HAC on trilostane may more clearly address the issues studied herein.

Footnotes

[a] Cortrosyn, Amphastar Pharmaceuticals Inc, Rancho Cucamonga, CA
[b] Cosyntropin, Sandoz Inc, Princeton, NJ
[c] Vetoryl, Dechra Veterinary Products, Overland Park, KS
[e] Immulite 2000 cortisol assay, Siemens Healthcare Medical Solutions, Los Angeles, CA
[d] Software: StatXact 11.0, Cytel Software Corporation, Cambridge, MA

Acknowledgments

The authors thank the technical staff at the Animal Specialty and Emergency Center, in particular Veronica Martinez, for their assistance in conducting this study. The authors also thank the owners and their dogs for their willingness to participate in this study.

Grant Support: This study was supported in part by generous donations from the late Ruth Johnston.

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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