Brief Communication
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Comparison of IV and IM Formulations of Synthetic ACTH for ACTH Stimulation Tests in Healthy Dogs

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Background: Two commercially available forms of synthetic ACTH are used to diagnose and monitor hyper- and hypoadrenocorticism in dogs.

Objective: To compare the biologic activity of the liquid and lyophilized forms of cosyntropin.

Animals: Eighteen privately owned healthy dogs were included.

Methods: Dogs were assigned to one of 2 groups of 9 dogs each. Group 1 dogs were tested with the lyophilized product first and the liquid solution 30–60 days later. The Group 2 dogs were tested with the liquid solution first and the lyophilized drug 30–60 days later. For the ACTH stimulation tests, serum samples were collected before and 1 hour after IM administration of 0.25 mg reconstituted lyophilized product or 1 hour after IV administration of 0.25 mg of liquid solution. Cortisol concentrations of all serum samples were measured by use of a commercial cortisol radioimmunoassay.

Results: Serum cortisol concentrations before and after ACTH stimulation did not differ significantly between groups ($P = .57$). In addition, no individual dog had as much as a 20% difference in serum cortisol concentrations after administration of either ACTH formulation.

Conclusions and Clinical Importance: Given the lack of significant differences of the ACTH stimulation test results, the lyophilized and liquid solution products can be used interchangeably.

Key words: Adrenal gland; Endocrinology; Hyperadrenocorticism.

Results of measuring circulating cortisol concentrations before and after adrenocorticotropic hormone (ACTH) administration are accepted as the "gold standard" for confirming or refuting the diagnosis of hypoadrenocorticism in dogs.[1,2] The ACTH stimulation test (ACTHST) is also widely accepted as an excellent means of assessing medical or surgical therapy for hyperadrenocorticism.[3–6] The ACTHST is frequently used as a screening test for hyperadrenocorticism in dogs, although sensitivity of test results has been questioned.[7,8]

Natural ACTH contains 39 amino acids. Since the introduction of synthetic ACTH to the veterinary literature in 1982, the synthetic preparation has gained popularity and continues to be commonly used in veterinary medicine.[9] That original product, referred to as "cosyntropin," is an α 1–24 corticotropin, a synthetic subunit of ACTH. It has been commercially available for almost 3 decades as a sterile lyophilized powder in vials containing 0.25 mg of cosyntropin and 10 mg of mannitol. Before lyophilization, the pH might be adjusted with acetic acid, sodium hydroxide, or both. It is recommended that the lyophilized powder be reconstituted with 1 mL of 0.9% sodium chloride and administered either by IV or intramuscular (IM) injection.[10]

Abbreviations:

ACTH	adrenocorticotropic hormone
ACTHST	ACTH stimulation test

Recently, a 1 mL liquid form of synthetic cosyntropin that does not require reconstitution has become commercially available. This sterile solution contains 0.25 mg of cosyntropin, 0.82 mg of sodium acetate trihydrate, 6.4 mg sodium chloride, 10 mg mannitol, 1 mg glacial acetic acid, and water.[11] The description of the amino acid sequence of each product is identical, suggesting that the biologic activity of the newer product should be similar to that of the previously described product. It is recommended that this liquid form of synthetic ACTH be for IV use only. The question raised when considering use of one versus the other product is whether or not their biologic activities are comparable.

If each drug causes maximal synthesis and secretion of glucocorticoids from canine adrenal cortices, then results of such tests would be interchangeable. Because both the lyophilized product and the solution product are used in veterinary medicine, it is warranted to compare the stimulatory nature of each drug to determine if test results are consistent.

Eighteen privately owned pet dogs were studied. No dog had received glucocorticoid medication in the previous year. This population included 12 castrated males and 6 spayed females. Eleven purebred dogs, including 2 Pointers and 9 other breeds, were represented, with the other 7 dogs being mixed breeds. The median age was 6 years old with a range of 1–10 years of age. Median body weight of this population was 18.5 kg with a range of 2–35 kg of body weight. Owners of each dog believed that their pet was healthy and each was considered healthy on physical examination. Each animal was further evaluated with a urinalysis, complete blood count, and serum chemistry profile. No

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abnormalities were identified on any test result in any of the dogs. Informed consent was obtained from the owners of all dogs studied.

The dogs were numbered, in order, 1 through 18 as they were placed into the study. Dogs with odd numbers were placed into Group 1 and dogs with even numbers were assigned to Group 2. Group 1 dogs were tested with the lyophilized Cortrosyn[a] first and the cosyntropin solution[b] 30–60 days later. The Group 2 dogs were tested with the cosyntropin solution first and the Cortrosyn 30–60 days later. For the ACTH stimulation tests, blood samples (2 mL each) were collected before and 1 hour after IM administration of 0.25 mg reconstituted lyophilized Cortrosyn. Alternatively, blood samples were collected before and 1 hour after IV administration of 0.25 mg of cosyntropin solution. All serum samples were separated and immediately frozen. All serum samples were batched and assayed together once all 18 dogs had received both products. Serum from all samples was assessed for cortisol concentration by use of a commercial cortisol radioimmunoassay that has been validated for use in dogs.[12] The analytic sensitivity of this assay was 0.3 μg/dL (8.3 nmol/L). The serum cortisol data were evaluated using analysis of variance for an experimental design in which there are repeated measures on the same experimental units. The specific repeated measures design involved is that described by Winer.[13] Computationally, the analysis was performed using BMDP2V.[14] All results are presented as mean ± SD Statistical significance was set at $P < .05$.

Serum cortisol concentration before ACTH administration in the 9 Group 1 dogs (2.6 ± 1.2 μg/dL) was not significantly different from the serum cortisol concentration in the 9 Group 2 dogs (2.4 ± 1.4 μg/dL; $P = .5$; reference range: 0.5–6.0 μg/dL). There was no statistically significant difference between the 1st baseline serum cortisol concentration from the Group 1 dogs (2.6 ± 0.8 μg/dL) when compared with the 1st baseline serum cortisol concentration from the Group 2 dogs (2.6 ± 1.0 μg/dL; $P = .5$), nor when comparing the 2nd baseline serum cortisol concentration from the Group 1 dogs (2.4 ± 0.8 μg/dL) with the 2nd baseline serum cortisol concentration from the Group 2 dogs (2.7 ± 1.3 μg/dL; $P = .5$). Also, there was no significant difference between the baseline serum cortisol concentrations from the 18 dogs before administration of IM Cortrosyn (2.7 ± 1.1 μg/dL) when compared with the baseline serum cortisol concentrations obtained from the 18 dogs before administration of IV cosyntropin (2.3 ± 1.4 μg/dL; $P = .3$). Serum cortisol concentration before ACTH administration from the 36 results obtained from all 18 dogs was 2.5 ± 1.2 μg/dL. All results were within the reference range.

Serum cortisol concentration after administration of ACTH in the 9 Group 1 dogs (13.1 ± 2.5 μg/dL) was not significantly different ($P = .6$) from the serum cortisol concentration in the 9 Group 2 dogs after administration of ACTH (13.0 ± 2.7 μg/dL; reference range: 5–17 μg/dL, 17–22 μg/dL "borderline," >22 μg/dL abnormal). There was no significant difference ($P = .5$) in serum cortisol concentration after IM ACTH administration in the Group 1 dogs (13.2 ± 2.7 μg/dL) when compared with the serum cortisol concentration after IV ACTH administration Group 2 dogs (13.3 ± 3.2 μg/dL). There was no significant difference ($P = .6$) in serum cortisol concentration after IV ACTH administration in the Group 1 dogs (12.8 ± 2.3 μg/dL) when compared with the serum cortisol concentration after IM ACTH administration in the Group 2 dogs (13.1 ± 2.4 μg/dL). Also, there was no significant difference in serum cortisol concentration from all 18 dogs after IM ACTH administration (13.1 ± 2.5 μg/dL) when compared with the serum cortisol concentration after IV ACTH administration in all 18 dogs (13.0 ± 2.7 μg/dL; $P = .6$). The mean serum cortisol concentration from all 36 results after administration of ACTH to all 18 dogs was 13.05 ± 2.62 μg/dL, with 34 of 36 results within the reference range and 2 values being 18.1 and 19.8 μg/dL. Thus, there was no significant difference in the adrenocortical reserve measured by the 2 stimulation products. There also was no significant effect of the order of method on the responsiveness of the adrenocortical reserve ($P = .6$). There was no evidence that any assumptions of the repeated measures analysis of variance method were violated. Adverse reactions were not seen in any dog during or after any testing.

It was arbitrarily assumed that a difference in post-ACTHST results $\geq$20% using the 2 formulations of ACTH would not be clinically acceptable. The absolute value of the mean ± standard deviation of the differences between the 2 formulations was 0.094 ug/dL ± 1.46 μg/ dL. To detect a 20% difference (ie, 2.6 μg/dL) in post-ACTHST serum cortisol concentration using the 2 formulations of ACTH, a sample size of 18 dogs was sufficient to achieve a statistical power virtually equal to 1. One of the 18 dogs had identical serum cortisol concentrations after administration of each ACTH formulation. Seven dogs had serum cortisol concentrations after administration of each ACTH formulation that differed by $\leq$1.0 μg/dL, 8 had differences of >1.0–<2.0 μg/dL, and 2 dogs had results which were 2.3 and 2.7 μg/dL different, respectively. In 9 dogs, the IM ACTH resulted in the higher serum cortisol concentration and in 8 the IV ACTH resulted in the higher serum cortisol concentration. Results of this study suggest that the 2 forms of ACTH used, as described, stimulate the adrenal cortices of healthy dogs similarly. Furthermore, if sick dogs respond to the products in the same way as healthy dogs, then the results of ACTHST using either product would be considered interchangeable.

Footnotes

[a] Cortrosyn, Amphastar Pharmaceuticals, Inc, Rancho Cucamonga, CA

[b] Cosyntropin, Sandoz Inc, Princeton, NJ

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