Evaluation of the use of baseline cortisol concentration as a monitoring tool for dogs receiving trilostane as a treatment for hyperadrenocorticism

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Objective—To determine whether a single measurement of cortisol concentration can be used to monitor dogs receiving trilostane for hyperadrenocorticism.

Design—Controlled drug efficacy trial.

Animals—103 client-owned dogs.

Procedures—Results of ACTH stimulation tests before and during trilostane treatment were evaluated. Each cortisol concentration after ACTH stimulation was classified as indicative of excessive, acceptable, or inadequate control of adrenal gland function, as outlined by the trilostane manufacturer. Baseline cortisol concentrations before and during trilostane treatment were evaluated; target variables were defined, and sensitivity, specificity, and predictive values were determined.

Results—Results of 103 and 342 ACTH stimulation tests before and during treatment were evaluated. In this population, baseline cortisol concentrations ≥1.3 μg/dL accurately excluded excessive suppression (defined by cortisol concentration after ACTH stimulation <1.5 μg/dL) in 254 of 259 (98%) dogs. In addition, baseline cortisol concentrations ≤2.9 μg/dL correctly excluded inadequate control (defined by cortisol concentration after ACTH stimulation >9.1 μg/dL) in 200 of 211 (95%) dogs. During trilostane treatment, baseline cortisol concentrations between 1.3 and either 2.9 μg/dL or ≤50% of the pretreatment baseline cortisol concentration correctly predicted acceptable control of adrenal gland function in 147 of 168 (88%) dogs.

Conclusions and Clinical Relevance—Evaluation of a baseline cortisol concentration collected 4 to 6 hours after trilostane administration in dogs with hyperadrenocorticism provided clinically useful information about control of adrenal gland function. Many dogs receiving trilostane may be adequately monitored without the expense and inconvenience of an ACTH stimulation test. (J Am Vet Med Assoc 2010;237:801–805)

Trilostane is a synthetic corticosteroid analogue that competitively inhibits 3β-hydroxysteroid dehydrogenase; this enzyme is required by the adrenal cortex for the synthesis of cortisol. Trilostane currently is approved for use in Europe, Australia, New Zealand, and the United States for dogs with hyperadrenocorticism attributable to a pituitary gland adenoma or an adrenal gland tumor. Periodic monitoring of adrenal gland function is required to prevent inadvertent hypocortisolemia and to ensure adequate control of cortisol release. Periodic ACTH stimulation tests are recommended so that appropriate adjustments can be made to the trilostane dose. The manufacturer of trilostane currently recommends a dose reduction when the cortisol concentration after ACTH stimulation is <1.45 μg/dL and a dose increase when the cortisol concentration after ACTH stimulation is >9.1 μg/dL. It requires at least 1 hour, 2 venipunctures, and 1 injection (IV or IM) to perform an ACTH stimulation test. A dog is usually allowed to remain with its owner or

**ABBREVIATIONS**

CI	Confidence interval
NPV	Negative predictive value
PPV	Positive predictive value

is placed in a cage while the test is performed. A monitoring method that could be completed without requiring that a dog be admitted to a veterinary hospital and with less patient stress and client expense may facilitate compliance with suggested monitoring protocols. The objectives of the study reported here were to investigate the relationship between baseline cortisol concentrations and cortisol concentrations after ACTH stimulation in dogs receiving trilostane and to attempt to define a range of baseline cortisol concentrations that could reliably be used to rule out the possibility of excessive or inadequate control of adrenocortical function.

**Materials and Methods**

**Animals**—A total of 103 client-owned dogs with naturally developing hyperadrenocorticism were used in the study. These dogs were part of a clinical trial to evaluate the efficacy of trilostane. Informed owner consent was obtained for all dogs. The protocol for the efficacy trial was reviewed and approved by the US FDA.

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Inclusion criteria for the efficacy trial included appropriate clinical signs, physical examination findings, and clinicopathologic data for hyperadrenocorticism, along with abnormal results on a low-dose dexamethasone suppression test (ie, cortisol concentration at 8 hours after dexamethasone administration, > 1.5 µg/dL). Dogs with tumors of the adrenal cortex were identified by use of abdominal ultrasonography. Pretreatment ACTH stimulation tests were performed in all dogs before the initiation of trilostane administration; dogs with pituitary-dependent hyperadrenocorticism were required to have a cortisol concentration after ACTH stimulation of > 19.9 µg/dL, whereas dogs with tumors of the adrenal cortex were required to have a cortisol concentration after ACTH stimulation of > 14.5 µg/dL. The cutoff value for dogs with tumors of the adrenal cortex was lower because a substantial proportion of dogs with this form of hyperadrenocorticism have a more moderate response to exogenously administered ACTH.10 Patients with diabetes mellitus, preexisting hepatic compromise, or renal failure were ineligible for the trial; in addition, dogs with distant metastasis of an adrenal gland tumor and that were considered unlikely to survive for at least 12 weeks were also excluded.

ACTH stimulation tests—A pretreatment ACTH stimulation test was performed on each dog < 15 days before starting administration of trilostane. The initial dosage of trilostane4 was determined on the basis of body weight in accordance with the manufacturer's published guidelines.8

As part of the efficacy trial, ACTH stimulation tests were performed on days 14, 28, 42, and 84 after initiation of trilostane treatment. Most of the dogs received trilostane once daily in the morning, but 14 of 103 dogs received doses of trilostane twice daily (morning and evening) during some part of the trial. All ACTH stimulation tests were started between 4 and 6 hours following administration of the morning dose of trilostane, and adjustments to the trilostane dose were determined by use of the cortisol concentration after ACTH stimulation, as described in the manufacturer's published guidelines.8

Each ACTH stimulation test was performed in the same manner. A blood sample was collected for measurement of the baseline cortisol concentration immediately before administration of a synthetic ACTH product.4 Each blood sample was placed in a serum separator tube, was allowed to clot at 20° to 25°C for 30 minutes, and then was centrifuged for 10 minutes at approximately 1,980 X g. Serum was harvested, transferred to a plain serum tube, and refrigerated at 2°C. The synthetic ACTH product was administered IV at a dose of 0.125 mg for dogs that weighed < 5 kg (< 11 lb) and 0.25 mg for dogs that weighed ≥ 5 kg. A blood sample for measurement of the cortisol concentration after ACTH stimulation was collected 60 minutes after ACTH injection and processed in the same manner as for the blood sample collected before ACTH administration.

Serum samples were shipped on ice via overnight delivery to a commercial veterinary reference laboratory.4 Cortisol concentrations were determined by use of a radioimmunoassay method that involved a gammacounter. A standard curve was created for each assay, and 3 concentrations of commercial (control) cortisol were included at the beginning and end of each assay. The laboratory reported cortisol concentrations to the nearest 0.1 µg/dL.

Statistical analysis—Data were analyzed by use of a commercial software program; results were considered significant at values of P < 0.01. When appropriate, data sets were tested for normality by use of the D'Agostino and Pearson omnibus normality test. Correlation between paired data points was determined by use of the Spearman method. Median values of selected data sets were compared by use of the Mann-Whitney U test for unpaired data. Reliability of novel hypotheses was tested by use of the Fischer exact method, with results and 95% CIs provided for sensitivity, specificity, PPV, and NPV.

Initially, the paired cortisol measurements for the ACTH stimulation tests performed 14 to 84 days after the start of trilostane administration were investigated for correlation. Subsequently, each cortisol concentration after ACTH stimulation was reviewed by use of the clinical trial guidelines, and control of cortisol release was classified as excessive (cortisol concentration > 1.5 µg/dL after ACTH stimulation), acceptable (cortisol concentration between 1.5 and 9.1 µg/dL after ACTH stimulation), or inadequate (cortisol concentration > 9.1 µg/dL after ACTH stimulation). Data pairs were then reviewed, and an attempt was made to identify a baseline cortisol concentration above which excessive suppression of cortisol synthesis could be safely excluded. Similarly, an attempt was made to identify a baseline cortisol concentration below which the possibility of inadequate control of cortisol synthesis could be safely excluded.

Comparisons between the pretreatment baseline cortisol concentrations and results of the ACTH stimulation tests while dogs were receiving trilostane were also performed. An attempt was made to define a percentage decrease from the pretreatment baseline cortisol concentration that might indicate acceptable control of adrenal gland function. This factor was then combined with the newly defined optimal range for baseline cortisol concentration, and the validity of this novel algorithm was statistically determined.

Results

A total of 342 ACTH stimulation tests performed after initiation of trilostane treatment were included in the data analysis. Baseline cortisol concentrations ranged from < 0.2 to 17.7 µg/dL (median, 2.4 µg/dL). Cortisol concentrations after ACTH stimulation ranged from 0.3 to 28.8 µg/dL (median, 4.7 µg/dL). Although the cortisol concentrations after ACTH stimulation typically were greater than the baseline cortisol concentrations, 29 (8%) cortisol concentrations after ACTH stimulation were less than the baseline cortisol concentrations. There was a significant correlation (r = 0.68; P < 0.001) between the baseline cortisol concentrations and the cortisol concentrations after ACTH stimulation, which confirmed a relationship between the 2 values (Figure 1).

A total of 103 pretreatment baseline cortisol concentrations were available for analysis. These ranged
from 2.1 to 19.8 μg/dL (median, 6.1 μg/dL). This value was significantly (P < 0.001) higher than the median value for baseline cortisol concentrations during treatment with trilostane.

Analysis by use of the manufacturer’s guidelines revealed that the cortisol concentration after ACTH stimulation indicated excessive suppression of adrenal gland function (ie, < 1.5 μg/dL) in 22 of 342 (6%) samples. The cortisol concentration after ACTH stimulation was within the range for acceptable adrenal gland function (ie, 1.5 to 9.1 μg/dL) in 255 of 342 (75%) samples. Inadequate control of cortisol release (ie, cortisol concentration > 9.1 μg/dL after ACTH stimulation) was detected in 65 of 342 (19%) samples.

Various values for the baseline cortisol concentration were compared with the cortisol concentration after ACTH stimulation (which represented the criterion-referenced standard) until a range with optimal utility was determined. Excessive suppression of adrenal gland function was best defined by a baseline cortisol concentration < 1.3 μg/dL. Eighty-three of 342 (24%) baseline cortisol concentrations were less than this value. When compared with the cortisol concentration after ACTH stimulation, this value had a significant (P < 0.001) NPV of 98% (Table 1). In all, 254 of 290 (98%) results were appropriately classified as inconsistent with excessive adrenal gland suppression by use of the cutoff value of < 1.3 μg/dL for the baseline cortisol concentration.

Inadequate suppression of adrenal function was best defined by a baseline cortisol concentration > 2.9 μg/dL. One hundred thirty-one of 342 (38%) baseline cortisol concentrations were higher than this value. When compared with the cortisol concentration after ACTH stimulation, this value had a significant (P < 0.001) NPV of 95% (Table 2). A total of 200 of 211 (95%) results were appropriately classified as inconsistent with adequate control of adrenal gland function by use of a cutoff value of > 2.9 μg/dL for the baseline cortisol concentration.

Therefore, the optimal range for baseline cortisol concentrations was defined as 1.3 to 2.9 μg/dL. In this study population, 128 of 342 (37%) baseline cortisol concentrations were within this range. Of these 128 results, 113 (88%) were correctly classified by use of this newly defined reference range (P < 0.001). Of the 15 incorrectly classified results, the cortisol concentration after ACTH stimulation indicated excessive suppression of the adrenal glands in 5 (4%) and inadequate control in 10 (8%) samples (Table 3). In addition, 8 dogs had a baseline cortisol concentration > 9.1 μg/dL and could have been appropriately classified as poorly controlled on this basis alone.

When the optimal range for the baseline cortisol concentration during trilostane treatment was defined as 1.3 μg/dL to either 2.9 μg/dL or ≤ 50% of the pretreatment baseline cortisol concentration (whichever was greater), almost half of the results (168/342 [49%]) were within this range (Figure 2). This range correctly indicated adequate control of adrenal gland function in 147 samples, which yielded a significant (P < 0.001) PPV of 88% (Table 4). In all, 147 of 255 (58%) dogs with acceptable control of adrenal gland function as determined on the basis of the cortisol concentration after ACTH stimulation could be identified by evaluation of

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**Table 1—Comparison of baseline cortisol concentration at a cutoff value of < 1.3 μg/dL against cortisol concentration after ACTH stimulation at a cutoff value of < 1.5 μg/dL as an indicator of adrenal gland suppression in dogs receiving trilostane for treatment of hyperadrenocorticism.**

<table>
<thead>
<tr>
<th>Baseline cortisol concentration</th>
<th>Cortisol concentration after ACTH stimulation</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1.3 μg/dL*</td>
<td>≤ 1.5 μg/dL†</td>
<td>263</td>
</tr>
<tr>
<td>≥ 1.3 μg/dL†</td>
<td>254</td>
<td>279†</td>
</tr>
<tr>
<td>Total</td>
<td>517</td>
<td>542</td>
</tr>
</tbody>
</table>

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**Table 2—Comparison of baseline cortisol concentration at a cutoff value of > 2.9 μg/dL against cortisol concentration after ACTH stimulation at a cutoff value of > 9.1 μg/dL as an indicator of adrenal gland suppression in dogs receiving trilostane for treatment of hyperadrenocorticism.**

<table>
<thead>
<tr>
<th>Baseline cortisol concentration</th>
<th>Cortisol concentration after ACTH stimulation</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 2.9 μg/dL*</td>
<td>≤ 9.1 μg/dL†</td>
<td>132</td>
</tr>
<tr>
<td>≤ 2.9 μg/dL†</td>
<td>200</td>
<td>211†</td>
</tr>
<tr>
<td>Total</td>
<td>332</td>
<td>342</td>
</tr>
</tbody>
</table>

*Considered indicative of a dose of trilostane that resulted in inadequate control of adrenal gland function. †Considered indicative of a dose of trilostane that did not result in excessive suppression of adrenal gland function. ¶For this row, PPV is 20% (95% CI, 12% to 31%). §For this row, NPV is 98% (95% CI, 96% to 99%). ‡For this column, specificity is 72% (95% CI, 70% to 74%). §For this column, sensitivity is 79% (95% CI, 75% to 84%).
the baseline cortisol concentration. Sixteen of 21 erroneous classifications were samples in which the cortisol concentration after ACTH stimulation was > 9.1 µg/dL. The other 5 erroneous classifications were dogs with an unexpectedly low cortisol concentration after ACTH stimulation (1.0 to 1.1 µg/dL) despite baseline cortisol concentrations (1.7 to 2.2 µg/dL) within the desired range.

**Discussion**

It is a general consensus that adrenocortical function should be monitored when dogs are receiving trilostane. There are 2 principal reasons for such monitoring: to avoid the risk of iatrogenic hypocortisolemia and to ensure adequate control of hyperadrenocorticism.

In the study reported here, we detected a significant correlation between baseline cortisol concentrations and cortisol concentration after ACTH stimulation measured 4 to 6 hours following trilostane administration in dogs with hyperadrenocorticism. In addition, a range for the baseline cortisol concentration that could be used to reliably indicate acceptable control of adrenal gland function was defined. Therefore, measurement of a timed baseline cortisol concentration may be regarded as a screening test for acceptable control of adrenal gland function; a result within the target range would preclude the need for an ACTH stimulation test in a substantial proportion of patients. For those dogs with baseline cortisol concentrations outside the defined target range, an ACTH stimulation test may still be necessary before appropriate adjustments in the dose of trilostane are made.

The manufacturer of trilostane currently recommends performing an ACTH stimulation test at 10 to 14 days after starting treatment (or after adjustment of the trilostane dose) and then another ACTH stimulation test at 28 days; tests should be repeated every 3 months thereafter. For some clients, achieving full compliance with these recommendations becomes a financial burden; consequently, visits to a veterinarian’s office for the monitoring tests may be postponed. In addition, a client needs to wait at the clinic for > 1 hour or to leave their dog and return again later. Either option may be less convenient than an outpatient visit and a single venipuncture, particularly if a validated and reliable in-house cortisol assay is available. Therefore, the authors suggest that use of a baseline cortisol concentration to assess the efficacy of trilostane treatment offers substantial benefits to both owners and patients. However, it is important to regard measurement of baseline cortisol concentrations as a screening test for adequate control of adrenal gland function in clinically stable patients; this technique is not designed to replace the criterion-referenced standard of an ACTH stimulation test when a more detailed evaluation of adrenal gland function is needed. Limitations of the use of baseline cortisol concentrations to monitor treatment should be carefully weighed by both pet owners and clinicians, and this option should possibly be reserved for patients in which the cost of an ACTH stimulation test is an issue for the owners.

Information regarding a patient’s overall health, including appetite, thirst, and frequency of urination, may substantially influence decisions regarding trilostane dosage. It would have been ideal to have collected information regarding a patient’s overall health, including appetite, thirst, and frequency of urination, and cortisol concentrations after ACTH stimulation (1.0 to 1.1 µg/dL) despite baseline cortisol concentrations (1.7 to 2.2 µg/dL) within the desired range.

**Table 3—Results of 342 ACTH stimulation tests performed on dogs with hyperadrenocorticism 4 to 6 hours after trilostane administration, categorized on the basis of a specified baseline cortisol concentration and the cortisol concentration after ACTH stimulation.**

<table>
<thead>
<tr>
<th>Baseline cortisol concentration</th>
<th>Cortisol concentration after ACTH stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1.3 µg/dL</td>
<td>1.5 to 9.1 µg/dL</td>
</tr>
<tr>
<td>-----------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>&lt; 1.3 µg/dL*</td>
<td>17</td>
</tr>
<tr>
<td>1.3 to 2.9 µg/dL†</td>
<td>5</td>
</tr>
<tr>
<td>&gt; 2.9 µg/dL†</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
</tr>
</tbody>
</table>

Values reported are the number of samples with the indicated cortisol concentrations.

*Considered indicative of a dose of trilostane that resulted in inadequate control of adrenal gland function.
†Considered indicative of a dose of trilostane that resulted in inadequate control of adrenal gland function.
‡Considered indicative of acceptable control of adrenal gland function.

**Table 4—Results of 342 ACTH stimulation tests performed on dogs with hyperadrenocorticism 4 to 6 hours after trilostane administration, categorized on the basis of either a specified baseline cortisol concentration or a value ≤ 50% of the pretreatment baseline cortisol concentration and the cortisol concentration after ACTH stimulation.**

<table>
<thead>
<tr>
<th>Baseline cortisol concentration</th>
<th>Cortisol concentration after ACTH stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 1.5 µg/dL*</td>
<td>1.5 to 9.1 µg/dL†</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>≤ 1.5 µg/dL*</td>
<td>17</td>
</tr>
<tr>
<td>1.5 to 2.9 µg/dL†</td>
<td>5</td>
</tr>
<tr>
<td>&gt; 2.9 µg/dL†</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
</tr>
</tbody>
</table>

See Table 3 for key.
cortisol concentration after ACTH stimulation, and clinical status could be examined. However, this was not part of the efficacy study protocol, and retrospective addition of the investigators’ clinical impressions would have been subject to substantial bias. Therefore, we are unable to comment on the clinical status of the 21 dogs with baseline cortisol concentrations within the target range but a low (< 1.5 µg/dL [n = 5 dogs]) or high (> 9.1 µg/dL [16]) cortisol concentration after ACTH stimulation.

Although we were unable to include clinical variables in the data analysis, the importance of this information should not be overlooked. Indeed, it appears likely that inclusion of such clinical data may help identify patients in which measurement of a baseline cortisol concentration is an appropriate screening test, thereby improving the usefulness of this approach. It would certainly be advisable to perform an ACTH stimulation test in any patient that was not well (anorectic, lethargic, vomiting, or diarrheic) or that had signs of hyperadrenocorticism. However, in a patient that had results of a physical examination within expected limits and a recent clinical history of normal thirst, urinary habits, and appetite, evaluation of a baseline cortisol concentration may provide sufficient information. On the basis of our findings, a healthy dog with a baseline cortisol concentration between 1.3 µg/dL and either 2.9 µg/dL or ≤ 50% of the pretreatment baseline cortisol concentration may safely continue to receive the current dose of trilostane. In addition, a baseline cortisol concentration ≥ 1.3 µg/dL could reliably exclude the possibility of an overdose of trilostane in an ill patient or when a patient is reevaluated following a reduction in the dose of trilostane.

We chose to use the manufacturer’s guidelines regarding the cutoff value for inadequate, acceptable, and excessive adrenal gland function for this study. However, there currently is a lack of consensus about the optimal range for the cortisol concentration after ACTH stimulation for dogs receiving trilostane, and various recommendations may be found in the veterinary literature.2,11 The guidelines we used may still be useful, but they may need to be modified on the basis of one’s definition of acceptable control.

We did not attempt to identify the small group of dogs that received trilostane twice daily and do not know whether this may have influenced our results and conclusions. It has been suggested that twice-daily administration of trilostane may improve clinical control and decrease the required total daily dose.7,11 Because both basal cortisol secretion and the response to administration of exogenous ACTH are proportional to plasma trilostane concentrations, it appears unlikely that variations in dosing frequency would substantially influence our findings. However, further studies may be necessary before this conclusion can be made with confidence. The timing of blood collection in relation to the administration of trilostane is much more likely to impact the value of our technique.

Results of the study reported here confirmed a relationship between baseline cortisol concentrations and cortisol concentrations after ACTH stimulation for dogs receiving trilostane. In addition, our findings suggest that a baseline cortisol concentration measured 4 to 6 hours after trilostane administration may be a useful monitoring tool in dogs with hyperadrenocorticism. Additional studies that incorporate subjective information about patient status (eg, thirst, urination, appetite, and coat condition) may help to clarify the clinical utility of this technique.

References