A comparison of once and twice daily administration of trilostane to dogs with hyperadrenocorticism*

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Key words
Hyperadrenocorticism, dogs, trilostane treatment

Summary
Objective: This retrospective study describes the use of trilostane given once versus twice daily in dogs with hyperadrenocorticism (SID vs. BID-group) in separate clinical trials. Material and methods: The groups were compared over a six month period using laboratory findings, dose required to suppress post-ACTH cortisol, and clinical scores from owner and clinician questionnaires. Results: Ninety-three dogs enrolled the trials but for analysis of the final visit results only 56 dogs filled the inclusion criteria: 30 dogs in the SID-group and 26 dogs in the BID-group. Both treatment groups showed an improvement in clinical scores with time and no significant difference between them. In the BID-group post-ACTH cortisol concentrations went below 250 nmol/l sooner and in a higher proportion of dogs than in the SID-group. Twice-daily administration of trilostane also achieved a faster and more effective control for comparable daily doses. A higher individual tolerability (based on clinical scores) was found in the SID-group but there were no supporting laboratory findings. No dogs developed serious side-effects. Conclusion: This study reveals only small practical differences between once and twice daily trilostane administrations in treating hyperadrenocorticism. And the overall benefits of twice daily dosing have to be considered against the effect on the owners and their compliance with treatment.

Schlüsselwörter
Hyperadrenokortizismus, Hunde, Trilostanbehandlung

Zusammenfassung

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Vergleich zwischen einmal und zweimal täglicher Applikation von Trilostan bei Hunden mit Hyperadrenokortizismus
Tierärzt Prax 2012; 40 (K): 415–424
Received: March 6, 2012
Accepted after revision: May 31, 2012

* This study was previously presented in abstract form at the Congress of the European College of Veterinary Internal Medicine (ECVIM) – Sept. 2008.
Introduction

Hyperadrenocorticism (HAC) is a common endocrine disease of dogs which causes a range of clinical signs including polyuria, polydipsia, polyphagia, lethargy and alopecia (11, 17, 18). It can be successfully controlled using trilostane (Vetoryl, Dechra Pharmaceuticals), which is a competitive inhibitor of the 3β-hydroxysteroid dehydrogenase 4,5-isomerase enzyme system thereby limiting cortisol production (5, 20, 22, 25, 27, 30). A starting dose of 3–6 mg/kg given once daily is recommended by the manufacturer who also provides monitoring guidelines. However, published data on optimal dose rate and frequency of administration remains limited. In one canine pharmacokinetic study, trilostane was rapidly absorbed in normal dogs, with a peak trilostane concentration 1.5–2 hours following administration which returned to baseline in about 18 hours, but there was a large inter-individual variability (Dechra Veterinary Products Limited, UK, data on file). Additionally, a significant difference between the cortisol responses in adrenocorticotropic hormone (ACTH) stimulation tests performed at 4 and 24 hours post-dosing has been demonstrated (1). Similarly the 8 hours post-ACTH cortisol concentration was significantly higher than the results after 2 hours in another study (29) suggesting that trilostane lasted a maximum of 13 hours with decrease in effect 8 to 9 hours of administration. Cortisol suppression for a maximum of 20 hours had also been established beforehand (1). These results suggested that at least some dogs might benefit from twice daily dosing.

The aim of this retrospective study was to describe the effect of trilostane when given once versus twice daily on the efficacy in controlling clinical signs and laboratory findings in dogs with hyperadrenocorticism as well as looking at the short-term side-effects of both treatment regimens. The dose required to achieve control as determined by cortisol value of post-treatment ACTH was also evaluated.

Materials and methods

Evaluated studies

In the process of obtaining marketing authorisation for trilostane for veterinary use two prospective but unblinded studies were performed. Dogs with signs suggestive of HAC were actively recruited at eight different veterinary centres in the United Kingdom between January 2001 and June 2002. The cases seen over the first 9 months were given trilostane once daily, and allocated to the SID-group. The cases seen subsequently were given trilostane twice daily, providing they were over 10 kg, and allocated to the BID-group. Dogs less than 10 kg continued to be included in the SID-group. This was necessary as the capsule sizes available at that time were not sufficiently small to allow twice daily dosing (and reformulation of drugs on trial was not permitted). The animals used for these studies remained in the trial for at least 24 weeks. These trials were run under an Animal Test Certificate issued by the Veterinary Medicines Directorate. Ethical approval for all procedures performed on these cases was obtained from a local ethics committee acting under guidance from the UK Home Office.

Information on age, sex, weight, breed, clinical history and physical examination was evaluated. Dogs with evidence of renal or hepatic failure, pregnant or lactating bitches, animals that had received trilostane and/or mitotane over the preceding 3 months and dogs with uncontrolled diabetes mellitus were excluded. Dogs were allowed concurrent drug therapy as long as it was not known to have a discernible effect on cortisol or ACTH concentration. The same diagnostic approach was used in each centre. Suspicion of HAC was based on history, clinical examination and routine blood analysis. The diagnosis of HAC was established by an abnormally high post-ACTH cortisol concentration above 600 nmol/l or by the failure to suppress cortisol concentration below 40 nmol/l 8 hours after administration of a low-dose of dexamethasone as described.

Table 1 Questionnaire to the veterinary surgeon: Disease severity and drug efficacy were scored as an analogue scale, coded for intensity of the clinical signs. Disease severity was based purely on the clinical signs. Drug efficacy was based on clinical signs presented. Tolerability of the trilostane was scored according to an overall evaluation of side-effects and adverse events.

<table>
<thead>
<tr>
<th>Questions</th>
<th>0 – poor</th>
<th>1 – satisfactory if some improvement in condition</th>
<th>2 – marked improvement in condition</th>
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<tr>
<td>Skin condition was assessed by the veterinarian using the scale of 0 – normal, healthy skin; 1 – slight changes; 2 – moderate changes; 3 – severe changes</td>
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<td>Disease severity was based purely on the clinical signs: 0 – no clinical signs; 1 – mild to moderate clinical signs; 2 – moderate to severe clinical signs</td>
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<td>Drug efficacy was based on clinical signs presented: 0 – poor if insignificant improvement in condition; 1 – satisfactory if some improvement in condition; 2 – marked improvement in condition</td>
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<td>Tolerability of the trilostane was scored according to an overall evaluation of side-effects and adverse events: 0 – poor; 1 – satisfactory; 2 – good</td>
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elsewhere (11, 17–19). Adrenal ultrasound and an endogenous ACTH assay value of less than 5 pg/ml (normal: 20–80 pg/ml) were used to identify adrenal dependent cases (14, 15). Haematology and biochemistry samples were submitted to one of two commercial laboratories (Royal Veterinary College or Nationwide Laboratories), depending on the veterinary centre preference. Cortisol was measured at a single external laboratory (Cambridge Specialist Laboratory Services) using a solid phase radioimmunoassay (Coat-a-Count, DPC) validated for the dog. Samples for endogenous ACTH analysis were sent to the same laboratory using cold packs and analysed using an immunoradiometric assay (ACTH IRMA; Nichols Institute) validated for the dog.

At 9–12 days (visit-2), 4 (visit-3), 12 (visit-4) and 24 weeks (visit-5) follow instigation of therapy, an ACTH stimulation test was started 2–6 hours post-dosing. A haematology and biochemistry profile was also obtained. An initial dose of trilostane based on weight range was prescribed: < 10 kg: 30 mg; ≥ 10 and < 20 kg: 60 mg; ≥ 20 and < 40 kg: 120 mg; ≥ 40 kg: 120–240 mg. This dose was given once daily (SID-group) or divided twice daily (BID-group) when capsule size would allow it, depending on the study trial. For each animal the lowest effective dose of trilostane was chosen and adjusted as necessary to achieve resolution of clinical signs and to maintain post-ACTH cortisol concentrations between 40–120 nmol/l (5, 25). The next available capsule size was used when the dose was changed (increase or decrease of ~50%) as it could not be split. The dose rate (dose/kg) and total daily dose (dose/kg/day) were calculated retrospectively.

Clinical progress was judged by comparing ACTH stimulation test results, clinicopathological findings as well as clinical improvement. Skin/coat condition; abdominal distension/girth; activity level, appetite, thirst, urination (quantity and frequency) and panting were assessed based on the owners and veterinary surgeon judgment by means of a questionnaire that was completed before treatment and on subsequent visits. Disease severity and drug efficacy were scored as an analogue scale, coded for intensity of the clinical signs. Tolerability of the trilostane was scored according to an overall evaluation of side-effects and adverse events (Tables 1 and 2).

Table 2 Questionnaire to the owner on the animal’s activity level, appetite, thirst, panting, and urination. In questions 2, 3, and 5 a score to one decimal place was allocated on a scale with 5 representing the normal grade.

Questions

Activity level
Please tick which ever of the following you feel most accurately describes your dog at the current time:
- very lethargic, sluggish and poor exercise tolerance
- slightly lethargic and drowsy
- normal activity level
- slightly more active than normal
- very active, always got plenty of energy

Appetite
Using the line below, please grade your dog’s current appetite. The middle of the line represents a normal appetite. Draw a vertical line at the appropriate point on the line.
(Anorexic ----------------------------------- ravenous appetite)

Thirst
Using the line below, please grade your dog’s current thirst. The middle of the line represents a normal drinking pattern. Draw a vertical line at the appropriate point on the line.
(Not drinking --------------------------------------- always drinking)

Panting
Please tick which ever of the following you feel most accurately describes your dog at the current time:
- hardly ever pants, even after exercise
- normal – only panting after walks and in hot weather etc.
- panting more than usual, not just after exercise

Urination – quantity and frequency
Using the two lines below, please grade your dog’s current quantity of urine expelled and secondly the frequency at which he/she passes urine. The middle of each line represents a normal urination pattern. Draw a vertical line at the appropriate point on the line.
a) When your dog urinates, generally, what quantity of urine is passed?
(Virtually no urine passed -------------- extremely large quantities passed).
b) How often does your dog urinate?
(Hardly ever passes urine ------------------ constantly passes urine)
Statistical analysis

Statistics analysis was carried out using SAS version-9. Any apparent pre-treatment differences between treatment groups were investigated using parametric or non-parametric tests as appropriate. Differences were considered significant at values of p ≤ 0.05. Analysis of the longitudinal data (visits 2–5) was carried out as well as an analysis of results at the final visit. Covariance pattern models (PROC MIXED) were used to compare treatment effects over the study period for continuous variables while general linear models (PROC GLM) were used to compare treatment effects on each of the continuous variables at the final visit. All questions on the owners’ questionnaire were scored with “normal” at the centre of the scale and divided into three categories: normal, high, low. Generalised Estimating Equations (using a SAS macro downloaded from The Mixed Models website) were used to analyse the longitudinal questionnaire scores while Generalised Logit Models (PROC LOGISTIC) were used to compare treatment effects on the scores at the final visit. Scores allocated by the veterinary surgeon for severity, skin and coat condition, efficacy and tolerability were treated as ordinal categorical data. Generalised Estimating Equations were again used to analyse the longitudinal questionnaire scores while Cumulative Logit Models (PROC GENMOD) were used to compare treatment effects on the scores at the final visit. The effect of treatment group on the probability of maintaining the treatment regime and dropping out prior to each visit was investigated using a logistic regression model (PROC LOGISTIC) with post-ACTH cortisol concentration and dose/kg as covariates.

Results

Patient selection

Ninety-three dogs started the trials at different centres. Since mixed models were used for the analysis of longitudinal data only four animals were completely excluded from all the analysis: one dog from group-SID for whom only pre-treatment data was available; and three dogs from the BID-group: one dog who came off treatment after 4 days; another dog whose initial dose was not reduced; and three dogs from the BID-group: one dog who came off treatment before the second visit. SID and BID trials comprised 39 and 30 dogs, respectively.

Seventy-two dogs (77.4%) had pituitary-dependent hyperadrenocorticism (PDH) (47/59 SID-group and 25/30 BID-group) and 17 dogs with adrenal-dependent hyperadrenocorticism (ADH) (12/59 SID-group and 5/30 BID-group). For analysis of the final visit results, a total of 56 dogs filled the inclusion criteria: 30 dogs in the SID-group (25/30 PDH; 5/30 ADH) and 26 dogs in the BID-group (21/26 PDH; 5/26 ADH). Approximately 41% dogs that started on SID-group did not complete the trial while only 18% of dogs in BID-group withdrew. The estimated odds ratio suggested that dogs in the SID-group were nearly 15 times as likely to drop out as those in the BID-group, with a 95% confidence interval. Clinical records were reviewed and no dogs withdrawn due to severe side-effects or a sudden deterioration in clinical condition. No significant differences were observed in age, gender and initial severity of disease between SID and BID-group. Mean age of SID-group was 10.3 years (SD = 2.0) and BID-group 9.7 years (SD = 2.2). However, given the inclusion limitations there was a significant difference in weight between groups, mean weight in the SID-group was 14.9 kg (SD = 8.7) and in the BID-group it was 24.8 kg (SD = 12.2) (p < 0.0001).

Haematology and serum biochemistry

Before treatment, mean pre-treatment haemoglobin concentration was greater for the BID-group 18.08 g/dl (SD = 1.96) than for the SID-group 16.89 g/dl (SD = 1.70) (p = 0.02). Over the remaining visits, mean haemoglobin concentrations were similar for both groups and within the reference range (12–18 g/dl). Mean haematocrit was within reference range (37–56%) for both groups at the pre-treatment and final visits and no significant differences were apparent between the groups. Mean lymphocyte counts were higher and variances greater for the BID-group over all the visits. At the final visit, the mean lymphocyte count for the SID-group was 1.15 × 10^9/l (SD = 0.56) whereas the BID-group was significantly higher at 1.72 × 10^9/l (SD = 0.85) (p = 0.002). There was no significant difference between treatment groups for the other haematological parameters.

Mean alkaline-phosphatase (ALKP) and alanine-aminotransferase (ALT) concentrations were significantly higher and variances greater for the SID-group over all the visits, and decreased over time for both groups, but there was no significant difference in ALT concentrations before treatment (p = 0.08). The greatest difference in mean ALKP concentration was pre-treatment when ALKP concentration of the SID-group was 2526 U/l (SD = 3102) and the BID-group was 809 U/l (SD = 637) (p = 0.001). Thereafter, ALKP concentrations in the SID-group decreased rapidly, levelling off from visit-4 onwards, while the BID-group decreased more slowly and variances for the BID-group appear more stable throughout. Mean ALT concentrations also decreased until visit-5 for the SID-group, while levelling off from visit-3 onwards for the BID-group. At the final visit, mean ALKP concentration was 851 U/l (SD = 1013) for the SID-group and 322 U/l (SD = 552) for the BID-group (p = 0.001); while mean ALT concentration was 138 U/l (SD = 185) for the SID-group and 75 U/l (SD = 85) for the BID-group (p = 0.04).

Mean bilirubin concentrations were consistently higher and variances greater, with many individuals outside reference range (0.0–5.0 μmol/l) in the SID-group, including pre-treatment. The difference in mean bilirubin concentration between both groups was statistically significant at all visits, although this difference decreased with time. By the final visit, mean bilirubin concentration was 4.7 μmol/l (SD = 0.68) for the SID-group and 2.7 μmol/l (SD = 0.38) for the BID-group (p < 0.001).
There were some particularly high values in the biochemistry profile for two animals, both in the SID-group. One dog had pre-treatment ALKP 19708 U/l, ALT 3381 U/l and bilirubin 191.9 μmol/l. All three parameters normalised after starting trilostane treatment. The same dog developed hyperglycaemia and glucosuria by visit-4 and was started on insulin therapy at that stage. Another dog had transient increase in bilirubin 174.5 μmol/l at visit-2. There was no other evidence of liver dysfunction, haemolysis or post-hepatic obstruction. This dog developed marked azotaemia by visit-5 (urea 51 mmol/l, creatinine 585 μmol/l and phosphorus 5.7 mmol/l). The data from both of these animals was only included in the analysis where the model fit permitted and the presence of the data did not affect the overall results of the analysis.

Before treatment calcium concentrations were not significantly different; thereafter, the SID-group remained fairly static while the BID-group showed an overall increase. By the final visit, there was a significant difference between mean calcium concentrations between the groups, SID-group was 2.46 mmol/l (SD = 0.03) (four animals had calcium values below the reference range), whilst for the BID-group was higher at 2.66 mmol/l (SD = 0.03) (p = 0.0001) with all dogs within the reference range (2.3–3.0 mmol/l). There was no significant difference between mean calcium concentrations between the two groups. Nonetheless, mean basal cortisol concentrations were reduced to below 250 nmol/l by visit-2 for the BID-group while the reductions were more gradual for the SID-group, taking until visit-4 to achieve results comparable with final levels. The greatest differences occurred during the first month of treatment [mean basal cortisol concentration 100.5 nmol/l (SD = 67.7) and 86.7 nmol/l (SD = 94.1) for SID-group at visits 2 and 3 respectively; whilst for BID-group mean basal cortisol concentration was 58.1 nmol/l (SD = 37.2) and 67.6 nmol/l (SD = 50.4) for the same visits (p = 0.17)]. By the final visit, mean basal cortisol concentration was greater for the SID-group [73.8 nmol/l (SD = 50.4)] than for the BID-group [64.1 nmol/l (SD = 47.9)], although this was not significant (p = 0.34).

The final mean post-ACTH cortisol concentration for all dogs was significantly lower [105.0 nmol/l (SD = 82.0)] than the pre-treatment value [798.1 nmol/l (SD = 329.8)] (p < 0.001) as expected because dose of trilostane was adjusted to achieve a target post-ACTH cortisol concentration. For both groups, there was a significant decrease by visit-2 in post-ACTH cortisol concentration and the overall mean was 172.4 nmol/l (SD = 134.0) with a

**Endocrine testing and cortisol concentrations**

There was no significant difference in basal cortisol concentrations between the two groups before treatment (Fig. 1). However, basal cortisol concentrations were reduced below 250 nmol/l by visit-2 for the BID-group while the reductions were more gradual for the SID-group, taking until visit-4 to achieve results comparable with final levels. The greatest differences occurred during the first month of treatment [mean basal cortisol concentration 100.5 nmol/l (SD = 67.7) and 86.7 nmol/l (SD = 94.1) for SID-group at visits 2 and 3 respectively; whilst for BID-group mean basal cortisol concentration was 58.1 nmol/l (SD = 37.2) and 67.6 nmol/l (SD = 50.4) for the same visits (p = 0.17)]. By the final visit, mean basal cortisol concentration was greater for the SID-group [73.8 nmol/l (SD = 50.4)] than for the BID-group [64.1 nmol/l (SD = 47.9)], although this was not significant (p = 0.34).

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### Adverse reactions

In the SID group the prevalence of side-effects was very low, with only one animal exhibiting transient hyperbilirubinaemia and mild gastro-intestinal signs. In the BID group side-effects were reported in five dogs and were considered mild-moderate severity. Signs included: anorexia (3), lethargy (3), vomiting (3) and soft-faeces/diarrhoea (2). No dogs developed severe side-effects requiring hospitalisation, or documented hypoadrenocorticism attributable to the trilostane during the period of the study.

### Response and tolerance to treatment

Based on questionnaires for owners and vets there was a significant improvement in all the clinical signs with time and no differences between treatments for any of the questions on the questionnaires.

- **Severity of the condition:** At visit-1, the mode for both groups was 2; scores decreased over the post-treatment visits with scores of zero (no clinical signs) being achieved from visit-2 for BID group and visit-4 for SID group. The mode at the final visit was 3 (normal) for both groups.

- **Efficacy of the treatment:** There was an improvement in efficacy scores over the visits for both groups with similar proportions of dogs showing good response at the final visit. For both groups there was an immediate decrease in appetite score for both groups and an immediate decrease in thirst score post-treatment, followed by a more gradual decrease over time. The score at the final visit for both groups was normal (3.33–6.67).

- **Activity level:** Pre-treatment, the mode for both groups was 2; scores increased over the post-treatment visits although, while scores for the SID group increased more gradually until the final visit, there was a levelling off of scores in the BID group from visit-3 onwards. The mode at the final visit was 3 (normal) for both groups.

- **Appetite and thirst:** There was an overall decrease in appetite score for both groups and an immediate decrease in thirst score post-treatment, followed by a more gradual decrease over time. The score at the final visit for both groups was normal (3.33–6.67).

- **Panting:** The proportion of ‘normal’ scores increased steadily for the SID group; while it increased more rapidly by visit-3 for the BID group and then levelled off. The mode at the final visit for both groups was 2 (normal).

- **Urination:** For both groups there was an immediate decrease in quantity of urination score post-treatment followed by a more gradual decline; while there was a continual decrease in frequency of urination scores, levelling off at the final; mean scores were comparable across all post-treatment visits. At the final visit both groups had normal score (3.33–6.67).

- **Skin condition:** There was little evidence of improvement in the skin condition score until visit-5 for the SID group and visit-4 for the BID group. Given maintenance of treatment, dogs from BID-group mean post-ACTH cortisol concentrations were within the target range from visit-2 onwards, while this was only achieved by the SID group by visit-5. At the final visit, the mean post-ACTH cortisol concentration was significantly greater for the SID group than for the BID group (SD = 2.6), respectively (p < 0.001).

**Dose required**

An overall increase in dose/kg/day for both treatment groups was seen over the course of the study (mean value of 5.7 mg/kg/day at the initial visit to 6.7 mg/kg/day at the final visit) ([Fig. 2](#)). At all visits, dose/kg/day and variances were greater for the SID group. Initially the SID group had a mean of 6.4 mg/kg/day (SD = 3.1) while the BID group had a lower mean of 4.4 mg/kg per day (SD = 1.4) (p < 0.0001). The mean final dose/kg/day was significantly greater for the SID group than for the BID group, 7.6 mg/kg/day (SD = 4.4) and 5.4 mg/kg/day (SD = 2.6), respectively (p < 0.001).

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Discussion

Previously published studies on trilostane have shown that it is an effective treatment for hyperadrenocorticism with similar survival times to those achieved with mitotane (1, 16). However studies on trilostane have used once or twice daily dosing according to authors or preference in study design. There are no studies directly comparing once versus twice daily trilostane apart from one abstract (8). The results provided in this paper were obtained from two clinical trials that were conducted sequentially at UK veterinary centres. Although dogs entering the trials were not formally randomised between treatment groups, they were allocated to treatment groups independent of their clinical status. The data presented in this paper was obtained between 2001/2002. The data was the property of Dechra Pharmaceuticals and was not released until trilostane was fully authorised for use in the UK in 2006. Double blinded studies directly comparing once and twice-daily administration are still required. However the data presented here provides important evidence for any effects of dose frequency on clinical efficacy.

The difference in weight and breed distribution between groups may have led to variations in laboratory results pre-treatment. The cause of the increased bilirubin concentrations in two of the dogs was never established and resolved rapidly. The degree of azotaemia and hyperphosphataemia in the other dog was likely caused by renal disease. One possible explanation for the development of overt azotaemia in this case may be the control of initial hypertension associated with HAC once trilostane was started. However, due to the retrospective nature of this study, urine specific gravity and blood pressure measurements were not available to confirm this hypothesis. Idiosyncratic drug reaction was considered less likely and idro-

![Fig. 2a](image1) Line graphs plotting the total daily dose from SID-group (black solid line) and BID-group (blue dashed line). X axis represents pre-treatment (visit-1) to final assessment (visit-5). At all visits, dose/kg/day and variances were greater for the SID-group. The mean final dose/kg/day was significantly greater for the SID-group than for the BID-group, 7.68 mg/kg/day and 5.42 mg/kg/day, respectively (p < 0.001). Significant differences between the groups at a given point are indicated (*).

![Fig. 2b](image2) Box-and-whisker plots depicting the total daily dose for the SID-group (grey boxes) and BID-group (white boxes). The numbers above the upper whisker represent the mean total daily dose (mg/kg). At all visits, mean total daily dose and variances were greater for the SID-group. The mean total daily dose was significantly greater for the SID-group than for the BID-group, 7.68mg/kg/day and 5.42 mg/kg/day, respectively (p < 0.001). Significant differences between the groups at a given point are indicated (*). Significant differences within the group over time that are identified by statistical analysis are described in the text.
Genic hypoadrenocorticism was excluded based on ACTH stimulation test results. Calcium concentrations were within reference range for both groups; however in the BID-group it was significantly higher by the final visit. Previous studies have demonstrated that calcium concentration increases with trilostane treatment, possibly associated with adrenal secondary hyperparathyroidism (24, 28).

The results showed that control of HAC was achieved more quickly, effectively and successfully (higher proportion achieved control) with the twice-daily administration despite using lower total daily dose. More effective control of HAC is supported by lower post-ACTH cortisol concentration and variances within the BID-group and also ALKP and ALT lower concentration by the final visit (18). There were no differences in clinical signs between treatment groups, apart from skin condition at the final visit. Nonetheless, there was a difference in how rapidly the clinical signs improved, with an earlier response for the BID-group. Their ALKP concentrations, pre and post-ACTH cortisol concentrations were lower and lymphocyte count higher than the SID-group. Therefore, the fact that the BID-group improved more rapidly and required an overall lower total daily dose to do so may reflect a less severe state of the disease at the outset. The fact that this difference was not apparent in the clinical signs recorded by the owners or veterinarians at the start of the treatment trial may be due to the subjectivity and relatively poor reliability of the analogue scales used in the questionnaires, minor differences being underestimated. A more rigorous assessment of clinical signs would have allowed a more useful comparison between treatment groups.

There were insufficient cases to analyse separately dogs with adrenal-dependent and pituitary-dependent HAC. But former analysis removing the adrenal dependent cases did not affect the conclusions drawn from the pituitary dependent cases. Also Helm and others (16) have shown a similar response to trilostane in dogs with adrenal-dependent HAC. Moreover, the underlying aetiology is not determined in many cases of hyperadrenocorticism so the data from adrenal-dependent cases was included.

Given that effective control of HAC should be based not just on laboratory results but also resolution of clinical signs, this study is the first attempt to evaluate the perceptions of owners and veterinarians of clinical control of HAC. Multi observer variability has to be taken into account; but those observations should still reflect a normal clinical setting. In almost every published study on trilostane the ACTH stimulation test has been the laboratory test of choice for monitoring trilostane therapy. However it has never been properly validated as the most appropriate test for this purpose. Other methods, such as measuring urine cortisol: creatinine ratio and acute phase protein concentrations have not been shown to be useful (2, 13). In contrast, Cook and Bond (9) suggested that basal cortisol concentration 4–6 hours after trilostane administration > 35.8 nmol/l and ≤ 80 nmol/l or ≤ 50% of the pre-treatment value was adequate to monitor therapy. Following this criteria, this study would suggest an appropriate control of HAC for the BID-group from visit-2 onwards, whereas for the SID-group it was only achieved by visit-4.

In this study ACTH stimulation tests were started 2–6 hours after trilostane administration. The trilostane doses were adjusted such that the post-ACTH cortisol concentrations were suppressed to 40–120 nmol/l (5, 23). The same cortisol concentration was aimed for both groups. Given maintenance of the treatment regimen, this study suggests that mean post-ACTH cortisol concentrations, as well as basal cortisol concentrations, for the BID-group reduced to their final values quicker than the SID-group and variances were smaller. More dogs were unable to complete the SID-trial but it appears that once-daily dose was better tolerated by those dogs that stayed on the trial. However this was the only evidence of a difference in tolerability and differences in laboratory findings were minor and therefore unconvincing.

Despite the decrease in appetite with the control of HAC, weight was maintained in most dogs along the study. As expected, there was a non-linear relationship with weight (with a particularly steep increase in dose/kg/day with decrease in weight at the very low weights, whilst at the higher weights the total daily dose rate tended to plateau). The mean final total daily dose was greater for the SID-group (7.6 mg/kg/day) than for the BID-group (5.4 mg/kg/day). Two dogs in the SID-group were less than 5 kg, which may have been slightly overdosed since the dose administered was dependent on the available capsule size. Dosing by capsule size and dose adjustments after only 9–12 days (visit-2) of trilostane administration is no longer recommended. The overall total daily dose in this study (6.7 mg/kg) is comparable to a previous study by Alenza and others (1), who recorded 6.5 mg/kg, but greater than mean final daily dose achieved by Vaughan and others (29) and also Feldman (12), both studies using twice-daily dosing, who recorded 3.8 mg/kg. Nonetheless, the dose in this study is still lower than those achieved in once-daily dosing studies from Neiger and others (20) 11.4 mg/kg and Braddock and others (5) 16.7 mg/kg. Further prospective randomised trials with different target ranges for post-ACTH stimulation cortisol concentration may be required for each treatment regimen and a more accurate assessment of clinical signs in order to compare minimal effective doses.

Animals on once-daily administration had a 15% increased chance of dropping out of the treatment. If a dog was not responding to the treatment then it would be switched to mitotane more readily, and the lack of initial response may have been a reason for withdrawal. Therefore this difference between SID and BID-groups may reflect the management of the trials rather than the drug itself. Also of interest was the number of dogs in the SID-group (13) with high and/or rapidly increasing dose rates that dropped out during the early stages of the clinical and were changed to twice-daily, presumably due to a lack of response to treatment. Similar rapid increases within the BID-group had the increased dose maintained until the final visit. When clinical records were reviewed, no dogs developed severe side-effects attributable to the trilostane. The low prevalence of side-effects is in agreement with previous studies.
Conclusion for practice

The administration of trilostane once vs. twice daily for the treatment of canine hyperadrenocorticism showed a clinical improvement for both groups based on owners and vets questionnaires but no significant differences between them. Nonetheless, twice-daily administration of trilostane achieved a faster and more effective control for comparable daily doses. Overall, this study reveals only minor differences between once and twice daily trilostane administrations in treating hyperadrenocorticism.

A recent comparison between two protocols using a low-dose twice-daily vs. once-daily dose administration showed a more rapidly control of clinical signs for SID but also a higher incidence of side-effects (8). Interestingly, two other papers (12, 29) have hypothesized that trilostane low-dose twice-daily would possibly resolve clinical signs while minimizing adverse reactions. Nonetheless, dogs still developed side-effects when given trilostane even for very short periods suggesting that other reasons rather than dose and/or frequency are responsible for the development of hypoadrenocorticism and adrenal necrosis (7, 10, 23, 26). Additionally, results from recent study (6) support the hypothesis that adrenal gland lesions seen in trilostane-treated dogs are caused by ACTH and not by trilostane itself.

Dogs were not evenly distributed between the two groups as there were differences in weight and laboratory findings pre-treatment, which is a key limitation of this study. However, this study reveals small differences between once and twice daily trilostane administrations in treating HAC, which is unlikely to be of practical significance. And the overall benefits of twice daily dosing have to be considered against the effect on the owners and their compliance with treatment.

Acknowledgements

The authors would like to acknowledge the generous provision of the data from the clinical trials by Dechra Pharmaceuticals and in particular Emma Arnold, Fran Holland and Susan Langhofer who were all involved at various stages of these studies. They would also like to acknowledge the involvement of the many veterinary surgeons, nurses and laboratories who were involved with the care of these patients and the recording of the data.

Declaration of conflict of interests

This study was initially funded by Dechra Pharmaceuticals. All the dogs in this study received free medication, clinical tests and consultations. No veterinarian directly involved in the care of these animals received personal financial benefit for participation in this study.
References


