Comparison of two treatment regimens with trilostane in dogs with pituitary-dependent hyperadrenocorticism

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Summary

Trilostane is used to treat dogs with pituitary-dependent hyperadrenocorticism (PDH). In our institution, it was initially dosed based on bodyweight (BW) categories, since April 06 it is dosed per kg BW. Our objectives were to compare effectiveness, number of dose adjustments and side effects of the two dose regimens in dogs with PDH. Dogs of group 1 (28 dogs) received trilostane based on BW categories (< 5 kg, 30 mg; 5 – 20 kg, 60 mg and > 20 kg, 120 mg; SID); dogs of group 2 (20 dogs) received 2 – 5 mg/kg SID. Treatment goal was a post-ACTH cortisol of 1 – 2.5 and 1.5 – 5.4 μg/dl in group 1 and 2, respectively. Starting doses were significantly higher in group 1 and stayed higher until re-check at 4 – 7 months. Baseline and post-ACTH cortisol were significantly decreased compared to pre-treatment at all time points in both groups. Significantly more dogs of group 2 (5/20) needed a dose increase at the first re-check and significantly more dogs of group 1 (10/23) a dose reduction at the last re-check. Intermittent discontinuation was necessary in 25 and 10 % of dogs of group 1 and 2, respectively. We conclude that dosing per kg BW results in comparable clinical improvement, decrease in cortisol, but lower risk of side effects.

Keywords: trilostane, dose regimens, hyperadrenocorticism, dogs

Introduction

Since several years trilostane, an orally administered competitive inhibitor of the 3β-hydroxysteroid dehydrogenase enzyme system is used to treat dogs with pituitary-dependent hyperadrenocorticism (PDH) (Potts et al., 1978). Trilostane has been shown to reduce the circulating concentrations of cortisol and lead to substantial improvement in clinical signs in 70 to 96 % of cases (Ruckstuhl et al., 2002; Braddock et al., 2003; Neiger et
The initial treatment regime was extrapolated from human medicine (Hurley et al., 1998), adjusted to the available capsule sizes and included: trilostane dosing based on 3 categories of bodyweights (BW) (< 5 kg, 30 mg PO, q24h; 5 to 20 kg, 60 mg PO, q24h; and > 20 kg, 120 mg PO, q24h) and treatment control with regular ACTH stimulation tests 2 – 6 hours after trilostane application with a target post-ACTH serum cortisol concentration between 1 – 2.5 μg/dl. In April 2006 the Vetoryl® consensus meeting was held in Amsterdam, Netherlands. Considering the experiences of the specialists and the recently available capsule sizes the following new dosing and monitoring guidelines were released: trilostane dosing per kg BW (2 – 5 mg/kg, PO, q24h) and treatment monitoring with ACTH stimulation tests 2 – 3 hours after trilostane application with a target post-ACTH serum cortisol concentration between 1.5 – 5.4 μg/dl. However, despite these resolutions, the package insert of Vetoryl® still recommends dosing based on BW categories. So far, no studies exist comparing the two treatment regimens in respect to their clinical efficacy or to their potential to induce side effects.

Besides the effect of trilostane to decrease plasma cortisol levels, trilostane was proven to lead to increases in cortisol precursor and in canine endogenous ACTH (cACTH) concentrations (Witt and Neiger, 2004; Sieber-Ruckstuhl et al., 2006). Furthermore, adrenocortical necrosis was documented histologically in several dogs treated with trilostane (Chapman et al., 2004; Reusch et al., 2007; Ramsey et al., 2008). The histologic changes were hypothesized to occur most probably because of the increase in cACTH concentrations. Prove for this hypothesis seemed to come from an experimental animal study, where rats treated with different doses of ACTH developed severe adrenal hemorrhage and vacuolization, whereas rats medicated with trilostane did not develop any adrenal changes (Burkhardt et al., 2008). Due to the possible disadvantageous effects of ACTH, its rise during trilostane therapy should be limited. Weather a lower trilostane dose results in less increase in cACTH than a higher dose would do, has not been evaluated. Therefore, objectives of this study were twofold: a) to compare the clinical effectiveness, the decrease of cortisol concentrations, the number of dose adjustments and the number of intermittent or permanent therapy withdrawal between the two dose regimens and b) to determine the cACTH concentrations in the two treatment groups.

** Animals, Material and Methods **

Group 1 consisted of 28 client-owned dogs, in which PDH was diagnosed between June 1999 and April 2006. Age ranged from 6 to 14 years (median, 9 years) and BW from 2.3 to 39 kg (median, 7.85 kg). There were 12 females (9 spayed) and 16 males (4 castrated). Breeds represented included Dachshund (n = 6), Crossbreed (5), Labrador Retriever (2), Parson Jack Russell Terrier (2), Poodle (2), Yorkshire Terrier (2), Bolognese (1), Golden Retriever (1), Hovawart (1), Italian Greyhound (1), Maltese (1), Pudelpointer (1), Scottish Terrier (1), Shihtz-Tzu (1) and Toy Poodle (1). Group 2 consisted of 20 client-owned dogs, in which PDH was diagnosed between May 2006 and December 2009. Age ranged from 6 to 14 years (median, 10.5 years) and BW from 5 to 38.6 kg (median, 10.35 kg). There were 13 females (11 spayed) and 7 males (2 castrated). Breeds represented included Crossbreed (6), Dachshund (3), Shihtz-Tzu (2), Bichon Frisé (1), Border Collie (1), Cairn Terrier (1), German Shepherd (1), Parson Jack Russell Terrier (1), Tibetan Terrier (1), Toy Poodle (1), West Highland White Terrier (1) and Yorkshire Terrier (1). All dogs underwent a thorough clinical examination. Blood and urine samples were collected for a CBC, biochemical profile, urinalysis and urine culture. Further work-up included a low-dose dexamethasone suppression test (LDDS), measurement of the urinary corticoid:creatinine ration (UCCR) and ultrasonographic examination of the adrenal glands. PDH was diagnosed on the basis of the dog’s concentration of endogenous ACTH and/or a symmetrical ultrasonographic appearance (with or without enlargement) of the adrenal glands. Dogs were included in the study when consistent clinical and laboratory findings for HC were present, the LDDS and/or the urinary UCCR were positive, the dog had not received other treatments (radiation treatment or mitotane) and the dog’s owner returned the dog to our clinical facility for regularly scheduled re-evaluations throughout a 6-month period.

** Diagnostic testing and hormone analysis **

The ACTH stimulation tests were performed by collecting blood samples for determination of serum cortisol before and 1 hour after intramuscular or intravenous injection of 0.25 mg of synthetic ACTH (Synachten®, Novartis Pharma Schweiz AG, Bern, Switzerland). Serum cortisol concentrations were determined by use of chemiluminescence assays (group 1: ADVIA Centaur® System, Bayer AG, Zürich, Switzerland; group 2: DPC Immulite® 1000, Siemens AG, Zürich, Switzerland). The LDDS test was performed by collecting blood samples before and 4 and 8 hours after injection of dexamethasone (Dexadreson®, Virbac AG, Küsnacht, Switzerland) 0.01 mg/kg intravenously. A cortisol concentration of ≥ 1.0 μg/dl in the sample collected 8 hours after dexamethasone administration was considered consistent with HC. UCCR measurements were performed on urine collected at home by the owners. Urinary corticoid concentrations were measured by use of a radioimmunoassay (RIA Beckmann, Unilabs Dr. Weber, St. Gallen, Switzerland). A UCCR of more than > 10 x 10⁶ was considered abnormal. Endogenous ACTH was determined before ACTH stimulation by collecting blood into chilled EDTA-coated tubes placed on ice. After centrifugation...
at 4 °C, plasma was stored at -80 °C until assayed. In group 1, measurement was performed at the University of Utrecht, The Netherlands, by use of a commercially available two-site immunoradiometric assay (IRMA, Nichols Institute, Wijchen, The Netherlands) (Bosje et al., 2002). In group 2, measurement was performed by a chemiluminescence assay (DPC Immulite® 1000, Siemens AG, Zürich, Switzerland). As AACTH concentrations in groups 1 and 2 were measured with different methods, only the relative (factorial) increase was compared between the two groups.

**Trilostane dose**

In the dogs of group 1, the initial dose of trilostane was determined on the basis of 3 categories of BW (< 5 kg, 30 mg PO, q 24h; 5 – 20 kg, 60 mg, PO, q24h; > 20 kg, 120 mg, PO, q24h). In the dogs of groups 2, the initial dose of trilostane was 2 – 5 mg/kg BW PO q24h. Thereby, dogs with a body weight < 5 kg were dosed in the lower dose range.

**Assessment of treatment control**

Efficacy of trilostane treatment was assessed by monitoring clinical signs and assessing results of ACTH stimulation testing. At each re-evaluation the clinicians were expected to assess the improvement in the general condition, the activity, the water intake, the frequency of urination, the appetite and the skin abnormalities according to the owner’s description.

ACTH stimulation tests were performed prior to trilostane treatment (t0) and after 1 – 2 (t1), 3 – 6 (t2), 7 – 15 weeks (t3), 4 – 7 (t4), and 8 – 12 months (t5) of trilostane treatment. At t1 – t5 the test was performed 2 – 6 h after the daily dose of trilostane in group 1 and 2 – 3 h after trilostane application in group 2. The treatment goal was to achieve a serum cortisol concentration of 1 – 2.5 µg/dl in group 1 and of 1.5 – 5.4 µg/dl in group 2, in samples obtained after ACTH stimulation. In dogs with post-ACTH cortisol concentration < or > than the target range, the trilostane dose was reduced or increased, respectively. The dose adjustments were made in increments of 5 – 20 mg/dog depending on the size of the dog.

**Statistical analysis**

Results were analyzed by means of non-parametric statistical methods (GraphPad Prism5, GraphPad Software, San Diego, CA, USA; SPSS 18.0 for Windows; SPSS Inc, Chicago, IL, USA). Ranges and median values are reported. Changes during the treatment with trilostane within groups were tested by use of Friedman’s repeated measures test and Dunn’s post test. Differences between the two groups were tested by the Kruskal-Wallis test and Dunn’s post test. Differences in dose adjustments, clinical signs and side effects were tested by the Chi-square test or the Fishers exact test, respectively. Differences were considered significant at values of p < 0.05.

**Results**

**Clinical signs**

Before starting trilostane therapy there were no significant differences in clinical signs between the two groups (p = 0.6 – 0.7). At t1, general improvement according to the owner had occurred in 18/22 dogs of group 1 and 18/20 dogs of group 2 (p = 0.44) (not significant). Significantly more dogs of group 1 (18/21 dogs) showed improvement of polyuria/polydipsia than dogs of group 2 (8/15) (p = 0.03). No significant difference was observed in improvement of polyphagia (group 1: 6/18; group 2: 3/7; p = 0.7), activity (group 1: 13/16; group 2: 6/10 dogs; p = 0.2), and skin abnormalities (group 1: 3/18; group 2: 1/8; p = 0.8) between the two groups. At t3 general improvement according to the owner had occurred in 22/23 dogs of group 1 and 17/17 dogs of group 2 (p = 0.4) (not significant). Significantly more dogs of group 2 (5/5) showed improvement of polyphagia than dogs of group 1 (9/20) (p = 0.03). No significant difference was observed in improvement of polydipsia (group 1: 19/23; group 2: 15/16; p = 0.3), activity (group 1: 16/18; group 2: 9/9; p = 0.3), and skin abnormalities (group 1: 12/21; group 2: 6/11; p = 0.9).

**Trilostane dose**

Starting doses of trilostane were significantly higher (p = 0.0001) in dogs of group 1 than in those of group 2 (Fig. 1). The median (range) initial doses were 6.1 mg/kg (3.0 – 13.0) and 3.8 mg/kg (2.0 – 4.3) in group 1 and 2, respectively. The trilostane doses stayed significantly higher in group 1 compared to group 2 until t4 (Fig. 1) (p = 0.007).

**Serum cortisol concentrations**

In both groups baseline and post-ACTH cortisol concentrations were significantly decreased at t1-t5 compared to t0 (group 1: p < 0.001; group 2: p < 0.05) (Fig. 2). No differences in baseline or post-ACTH cortisol values were found between the 2 groups at any time points (baseline cortisol: p = 0.1 – 0.7; post-ACTH cortisol: p = 0.1 – 1.0).

**Endogenous ACTH concentrations**

In both groups, there was no significant increase in endogenous ACTH concentrations after starting trilostane therapy (group 1: p = 0.6; group 2: p = 0.09). There was no significant difference in the relative increase of endogenous ACTH concentrations at any time point between the 2 groups (p = 0.5 – 0.9).
Dose adjustments

The total number of recorded dose adjustments during the study did not differ between the two groups (group 1: dose increase: 16, dose decrease: 28, no change of dosage: 56; group 2: dose increase: 23, dose decrease: 25, no change of dosage: 52; \( p = 0.5 \)). The trilostane dose had to be increased significantly more often at t1 in dogs of group 2 (5/20), than in dogs of group 1 (3/28) (\( p = 0.049 \)). At t5 the dose had to be decreased significantly more often in the dogs of group 1 (10/23), than in the dogs of group 2 (2/8) (\( p = 0.014 \)).

Withdrawal of trilostane due to possible side effects

Due to possible side effects (e.g. reduced appetite, vomiting) trilostane had to be intermittently withdrawn in 7/28 (25%) dogs of group 1 and 2/20 (10%) dogs of group 2. This difference was not statistically significant (\( p = 0.2 \)). In group 1, trilostane was stopped twice (at t1 and t3) in one dog, because of diarrhea, vomiting, anorexia, lethargy, hyperkalemia and low post-ACTH cortisol concentrations. The dog was started intermittently on mineralocorticoid and glucocorticoid supplementation for 3 days. After 7 days trilostane was restarted with half the original dose. In 4 dogs, trilostane was stopped (between t1/t2, t2/t3 or around t5, respectively) for several days to one week, because of clinical signs possibly related to cortisol deficiency (reduced general condition (2), diarrhea (1), reduced appetite (2), lethargy (1)). No ACTH-stimulation test was available. Trilostane was restarted with the same dose (3) or with two third of the original dose (1). In two dogs, trilostane withdrawal was based on owner decision only (between t2/t3 and t3/t4, respectively). In both dogs, trilostane was restarted by the owner in the same dose (after 1 week and 1.5 months, respectively).

In group 2, trilostane was stopped intermittently in 2 dogs at t1 and t3, respectively. Both dogs were in a good clinical condition with no signs of cortisol deficiency. Baseline and post-ACTH cortisol concentrations, however, were below the detection limit of the assay in either dog. In dog one, trilostane was restarted after 3 days with a third of the original dose; in dog two, trilostane was restarted after 8 days with half of the original dose. In both groups, trilostane was withdrawn permanently in 1 dog. Both withdrawals were based on owner decisions. The dog of group 1 seemed more tired than usual and the owner decided to stop trilostane around t4. No ACTH stimulation test was available. The dog of group 2 had several diseases (hyperadrenocorticism, hyperparathyroidism, mammary tumours, Horner syndrome) and the owner decided to stop trilostane even though the post-ACTH cortisol concentration was within the optimal treatment range.

![Figure 1: Trilostane doses at different time points. Scatter plots of trilostane dose (mg/kg) in dogs of group 1 (*) and of group 2 (†) before (t0) and 1 – 2 weeks (t1), 3 – 6 weeks (t2), 7 – 15 weeks (t3), 4 – 7 months (t4) and 8 – 12 months (t5) after starting trilostane therapy. The small numbers in parenthesis above the data points represent the number of dogs at each re-evaluation.](image-url)
One purpose of the present study was to compare the effectiveness of 2 different trilostane dose regimens to treat dogs with PDH. Application of trilostane per kg BW resulted in significantly lower starting doses compared to administration per BW categories. The total trilostane dose in group 2 remained significantly lower until the re-evaluation at 4–7 months, even though it was increased more frequently at the first re-evaluation. Most likely, the higher starting dosages in group 1 are responsible for the slightly faster improvement of polyuria/polydipsia documented after 1–2 weeks of therapy. However, already after 3–6 weeks of treatment this difference had disappeared, while the dosages were still significantly different. This clearly shows that administering trilostane per kg BW is comparably efficient, but that until the full response is reached more time is necessary. Recently, two different trilostane dose regimens were compared in dogs < 5 kg (Cho et al., 2013). One group of dogs was treated with a low trilostane dose (0.78 ± 0.26 mg/kg) twice daily, while the other group of dogs was treated with 30 mg/dog once daily. Similar to our study, they showed a slower onset of clinical improvement with the lower trilostane dose, but no difference in clinical improvement at the end of the study (after 24 weeks). In addition, a tendency for lower side effects with the low-dose regimen was reported.

Baseline and post-ACTH cortisol concentrations are known to decline significantly during trilostane therapy (Sieber-Ruckstuhl et al., 2006; Galac et al., 2010). Similarly in our study, cortisol concentrations (baseline and post-ACTH) were significantly decreased at all time points in both groups. Moreover, cortisol levels were not different between the two groups, which conclusively indicates, that dosing trilostane per kg BW is equally efficient as dosing per BW categories. Although the target area of post-ACTH cortisol was lower in group 1 than in group 2, the clinical improvement after 7–15 weeks of trilostane therapy was similar. This indicates that the
higher cortisol target area is sufficient to induce a good clinical control.

Owing to reduced appetite or vomiting, trilostane had to be intermittently withdrawn in 25% of dogs of group 1 and 10% of dogs of group 2. Although this difference in intermittent discontinuation was not statistically significant, there’s a tendency for fewer side effects with the low-dose trilostane regime. One can speculate that with a higher number of cases the distinction would have been significant. Thus, the tendency to overdose dogs with trilostane seems to be higher if it is dosed based on BW categories and if the lower post-ACTH target range is used. Unfortunately, in most dogs with intermittent trilostane withdrawal, no ACTH stimulation test could be performed to confirm trilostane overdose. On one side, this is due to the retrospective nature of the study and on the other side, due to the owner deciding themselves to stop trilostane in case of reduced general condition of their dogs.

Endogenous ACTH concentrations increase during trilostane therapy; a reflection of the diminished negative feedback of cortisol to the pituitary gland and the hypothalamus (Witt and Neiger, 2004; Sieber-Ruckstuhl et al., 2006; Galac et al., 2010). The hypothesis that a lower trilostane dose would lead to a lesser increase in cACTH could not be proven in the present study. Explanations for this could be: First, a pulsatile release pattern of cACTH, so that a single determination would lead to highly variable results; second, a correlation between the rise in ACTH and the size of the pituitary tumour. A positive correlation between the size of the pituitary tumour and the cACTH concentrations at the time of diagnosing HC has already been documented (Kipperman et al., 1992; Kooistra et al., 1997). To further evaluate these points, serial daily measurements of cACTH during trilostane therapy and comparison with the size of the pituitary gland should be done.

One drawback of the present report is that, due to the retrospective nature of the study, differences between the two groups could exist. At the time when the dogs in group 2 presented themselves with HC, HC was already a well-known syndrome. For this reason, owner possibly presented their dogs earlier to a veterinarian and dogs suffered from a milder form of disease. Another limitation of all the studies about trilostane therapy is the lack of consensus on the best time point for blood sampling during trilostane therapy and on the optimal concentration. Despite the mentioned drawbacks, we think that this study offers important findings for the further treatment of dogs with HC and encourages the appropriateness of the resolutions of the consensus meeting.

In summary, application of trilostane per kg BW leads to significantly lower starting doses and lower doses until the re-evaluation after 4–7 months than administration per BW categories. However, even though dosages are lower and the post-ACTH cortisol target range is higher, clinical improvement and decrease in cortisol concentrations were similar. In addition, there seems a tendency for lower side effects and less trilostane withdrawal when doses per kg BW are used. Finally, as cACTH concentrations did not differ between the two treatment groups, it can be concluded that cACTH probably is not influenced by the trilostane dosage.

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Comparaison de deux schémas de traitement de l’hyperadrénocorticisme hypophysaire chez le chien au moyen du trilostane

Le trilostane est en Suisse le seul médicament enregistré pour le traitement de l’hyperadrénocorticisme hypophysaire. Dans les débuts, le trilostane a été dosé dans notre clinique selon les catégories de poids; depuis avril 2006 nous le dosons en fonction du poids exact. Le but du présent travail était de comparer l’efficacité, le nombre d’ajustement de la dose et les effets secondaires des deux schémas de dosage chez des chiens souffrant d’hyperadrénocorticisme hypophysaire. Chez les chiens du groupe 1 (28 chiens), le dosage à été fait de la façon suivante: < 5 kg, 30 mg; 5 – 20 kg, 60 mg; > 20 kg, 120 mg; q24h. Les chiens du groupe 2 (20 chiens) recevaient 2 – 5 mg/kg q24h. Le but du traitement était d’atteindre un taux de cortisol après ACTH entre 1 et 2.5 ug/dl dans le groupe 1 et entre 1.5 – 5.4 ug/dl dans le groupe 2. Les doses initiales étaient significativement plus hautes dans le groupe 1 et restaient plus élevées jusqu’au contrôle après 4 à 7 mois. Les taux de cortisol basal et après ACTH étaient significativement plus bas par rapport à ceux mesurés avant le traitement dans les 2 groupes, et ce à tout moment. La dose a du être augmentée lors du premier contrôle de façon significativement plus fréquente (5/20) dans le groupe 2. La dose a du être réduite lors des derniers contrôles de façon significativement plus fréquente (10/23) dans le groupe 1. Des interruptions de courte durée du traitement ont été nécessaires chez 25 respectivement 10 % des chiens des groupes 1 respectivement 2. Le dosage du trilostane en fonction du poids en kilo amène une réponse thérapeutique et une chute du taux de cortisol comparables, mais avec moins d’effets secondaires.

Confronto tra due schemi terapeutici a base di trilostano per la cura dell’iperadrenocorticismo ipofisario nel cane

Il trilostano in Svizzera è l’unico medicinale omologato per il trattamento dell’iperadrenocorticismo ipofisario (HA). All’inizio, nella nostra clinica, il trilostano è stato dosato per categoria di peso; dall’aprile 2006 viene dosato per kg di peso corporeo. Scopo del presente studio è di paragonare l’efficacia, il numero delle modifiche posologiche e gli effetti secondari dei due schemi di dosaggio nei cani affetti da iperadrenocorticismo ipofisario (HA). Nei cani del gruppo 1 (28 cani), il trilostano è stato dosato nel modo seguente: < 5 kg, 30 mg; 5 – 20 kg, 60 mg; e 20 kg, 120 mg; q24h. I cani del gruppo 2 (20 cani) hanno ricevuto 2 – 5 mg/kg q24h. L’obiettivo del trattamento era di ottenere un valore post ACTH del cortisolo compreso tra 1 – 2.5 ug/dl nel gruppo 1 e tra 1.5 – 5.4 ug/dl nel gruppo 2. Le dosi iniziali erano significativamente più elevate nel gruppo 1 e sono state modificate fino al controllo dopo 4 – 7 mesi. I valori di cortisolo basale e post ACTH erano significativamente inferiori rispetto a quelli prima dell’inizio della terapia in entrambi i gruppi e in ogni momento. Da rilevare nei cani del gruppo 2 (5/20) che si è dovuto aumentare il dosaggio al 1° controllo. Da rilevare nei cani del gruppo 1 (10/23) che si è dovuto diminuire il dosaggio all’ultimo controllo. Un’interruzione prematura è stata necessaria nel 25 % e nel 10 % dei cani del gruppo 1 e 2. Un dosaggio del trilostano per kg di peso corporeo ha condotto a una risposta clinica comparabile e a una caduta del livello di cortisolo, con minori effetti secondari.

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