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Efficacy of Low- and High-Dose Trilostane Treatment in Dogs (< 5 kg) with Pituitary-Dependent Hyperadrenocorticism

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Background: Trilostane is commonly used to treat pituitary-dependent hyperadrenocorticism (PDH) in dogs. There are differing opinions regarding the dose and frequency of trilostane administration in dogs with PDH.

Objectives: To compare the efficacy of 2 trilostane protocols in the treatment of dogs with PDH.

Animals: Sixteen client-owned dogs with PDH and a body weight <5 kg.

Methods: Prospective observational study. Group A (n=9; low-dose treatment group) received 0.78 ± 0.26 mg of trilostane/kg PO every 12 h and group B (n = 7; high-dose treatment group) 30 mg of trilostane/dog PO every 24 h. All of the dogs were reassessed at 2, 4, 8, 12, 16, and 24 weeks after the initiation of treatment.

Results: An improvement in both ACTH-stimulated serum cortisol concentrations and clinical signs occurred more slowly in group A than in group B; however, after 20 weeks of treatment, 2/7 dog in group B had clinical signs and abnormal laboratory findings consistent with hypoadrenocorticism. At 24 weeks, an improvement in the clinical findings of all of the dogs in both groups was detected.

Conclusions and clinical importance: In dogs with PDH, twice-daily administration of low-dose trilostane is an effective approach to the management of PDH. In addition, our results suggest fewer potential adverse effects if trilostane is administered twice daily in the lower dose.

Key words: Adrenocorticotropic hormone; Cortisol; Cushing's disease; Dogs.

Introduction

Hyperadrenocorticism (HAC) is a common endocrinopathy that occurs in middle-aged to older dogs. Its clinical signs result from an excess of endogenous cortisol[1] and commonly include polyuria, polydipsia, polyphagia, dermatologic abnormalities, abdominal distension, and lethargy.[2] It is estimated that approximately 85% of dogs with HAC have a pituitary-dependent form (PDH), while 15% have an adrenal-dependent form.[3,4]

PDH in dogs is mostly treated medically, although there are other options, including surgery and radiotherapy.[5,6] Among the drugs commonly used in the treatment of PDH is mitotane (o,p-dichlorodiphenyldichloroethane), which has good efficacy but also potential adverse effects as well as disadvantages including transient hypoadrenocorticism, permanent mineralocorticoid and glucocorticoid deficiencies, drug intolerance, and a high frequency of relapse.[7] Ketoconazole and L-deprenyl also have been investigated for the treatment of PDH.[8–11] The former is an imidazole derivative that interferes with cortisol synthesis by inhibiting cytochrome-P-450-dependent enzymes. In the few dogs thus far treated with ketoconazole, positive results have been obtained in the majority, but larger studies are needed and there are additional concerns regarding the potentially hepatotoxic effects of the drug.[9] L-deprenyl, a monoamine-oxidase inhibitor, has been approved for the treatment of dogs with PDH in some countries, but is of limited efficacy.[11,12]

Recently, trilostane (4α,5-epoxy-17ß-hydroxy-3-oxo-5α-androstane-2α-carbonitrile) has gained increasing acceptance in the treatment of dogs with PDH, and its efficacy has been reported in several studies.[13,14] As a competitive inhibitor of 3β-hydroxysteroid dehydrogenase, trilostane inhibits the synthesis of several steroids in the adrenal cortex, including glucocorticoids and mineralocorticoids.[15,16] It appears to have fewer adverse effects than mitotane[13,17–19]; however, it might give rise to anorexia, vomiting, diarrhea, and weakness in dogs with PDH.[13,17,20] Furthermore, the dose of trilostane and the frequency of its administration as recommended by its manufacturer do not result in consistent suppression of serum cortisol concentrations over a 24-h period.[14] A protocol in which trilostane is administered to dogs every 8 or 12 h has been examined.[21,22] In the controlled study described herein, the efficacy and safety of 2 other trilostane protocols, a twice-daily low-dose protocol and a once-daily high-dose protocol, were

Abbreviations:

ACTH	adrenocorticotropic hormone
ADH	adrenal-dependent hyperadrenocorticism
ALP	alkaline phosphatase
ALT	alanine aminotransferase
HAC	hyperadrenocorticism
LDDST	low-dose dexamethasone suppression test
PDH	pituitary-dependent hyperadrenocorticism
SD	standard deviation

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compared with respect to their efficacy and safety in the treatment of dogs with PDH.

Materials and Methods

Animals

Twenty-five client-owned dogs newly diagnosed with PDH between May 2010 and April 2011, and 16 dogs weighing <5 kg and without concurrent disease were included in the study. The Chungbuk National University Animal Ethics Committee approved all animal use, and informed consent was obtained from the owners. The dogs were randomly divided into 2 groups, which differed in terms of the trilostane[a] dose and the frequency of its administration. Dogs in group A (n = 9) were treated with low-dose trilostane (0.78 ± 0.26 mg/kg) administered twice daily, while dogs in group B (n = 7) were treated with high-dose (30 mg/dog) trilostane once daily.

A tentative diagnosis of HAC was based on the history, physical examination findings, hematological results, biochemical profiles, and urinalyses. Biochemical profiles including alkaline phosphatase (ALP), alanine aminotransferase (ALT), total cholesterol, triglyceride, and glucose concentrations were determined using an auto-analyzer.[b] Electrolyte values, including sodium and potassium concentrations, were assayed using an electrolyte analyzer.[c] If the history and physical examination appeared suspicious, the dog underwent further clinicopathologic studies, including a complete blood count, biochemical profile, and urinalysis. The main signs noted by the owners were excessive urination and drinking, which are consistent with polyuria and polydipsia, and each dog showed at least 4 of the following 7 clinicopathologic findings: high serum alkaline phosphatase activity, high serum alanine aminotransferase activity, hypercholesterolemia, hypertriglyceridemia, hyperglycemia, and urine specific gravity <1.020. In addition, each dog underwent adrenocorticotropic hormone (ACTH) stimulation testing and low-dose dexamethasone suppression tests (LDDSTs). All of the dogs were also examined by abdominal ultrasonography.[d] PDH was differentiated from a functional adrenal tumor based on the results of the LDDST and abdominal ultrasonographic evaluations of the adrenal glands as described elsewhere.[2,3,22]

Endocrine Test

For ACTH stimulation testing, blood samples were collected before and 1 hour after the intravenous administration of synthetic ACTH[e] (0.25 mg/dog). Serum cortisol concentrations were measured using a chemiluminescent immunoassay-based auto-analyzer.[f] HAC was concluded based on an exaggerated increase (>22 μg/dL) in the ACTH-stimulated serum cortisol concentration.[22] At each reassessment after the initiation of trilostane treatment, all the dogs in groups A and B underwent an ACTH stimulation test 3–4 h after trilostane administration. For the LDDST, blood samples were collected before and at 4 and 8 h after intravenous administration of dexamethasone (0.01 mg/kg).[g] Dogs with cortisol suppression at 4 h and those with high cortisol at 8 h were considered to have PDH.[2,3] Suppression was defined as a serum cortisol concentration of < 1.5μg/dL 4 hours after dexamethasone administration or serum cortisol concentration <50% of the baseline concentration 4 or 8 hours after dexamethasone administration.[2,3]

Treatment Protocols and Assessments

The owners were requested to bring their dogs in for reassessment at 2 weeks, and then again at 4, 8, 12, 16, and 24 weeks after the initiation of trilostane treatment. To assess the response to treatment, owners were interviewed regarding changes in their dog's clinical signs and any adverse effects of treatment. At follow-up, parameters including polydipsia/polyuria, polyphagia, abdominal size and dermatologic problems, including alopecia, were evaluated. A physical examination, biochemical analysis, urinalysis, and ACTH stimulation test were also performed at every reassessment.

In group A (low-dose trilostane protocol), the initial trilostane dose was 0.5–1.0 mg/kg, administered PO every 12 h. Group B (high-dose trilostane protocol) received 30 mg of trilostane/dog, administered PO every 24 h. All of the dogs in both groups received trilostane in their food. The target range for the ACTH-stimulated serum cortisol concentration was 2–5.5 μg/dL.[21] The initial dose given to each dog was maintained for 4 weeks regardless of clinical signs including polydipsia, polyuria, and polyphagia and ACTH-stimulated serum cortisol concentrations >5.5 μg/dL. However, at serial reassessments beginning at 4 weeks, the trilostane dose was adjusted accordingly. If the dogs had an improvement or disappearance of clinical signs and an ACTH-stimulated serum cortisol concentration between 2 and 5.5 μg/dL, the trilostane dose was maintained as the final dose. In dogs responding well to treatment but with a high ACTH-stimulated cortisol concentration (>5.5 μg/dL), the trilostane dose was also left unchanged. However, if dogs with an ACTH-stimulated cortisol concentration between 5.5 and 9.0 μg/dL still showed clinical signs, the trilostane dose was increased by 25%. Dogs with clinical signs and an increased ACTH-stimulated cortisol concentration >9.0 μg/dL were shifted to a 50% higher dose of trilostane.[17] If the dogs showed an improvement in clinical signs and an ACTH-stimulated cortisol concentration of <2 μg/dL, the trilostane dose was decreased by 25–50%. On the other hand, if the dogs showed clinical signs consistent with hypoadrenocorticism and an ACTH-stimulated cortisol concentration <2 μg/dL, trilostane was discontinued and then restarted at a 50% lower dose after 2 weeks.

Statistical Analyses

All statistical analyses were carried out using a commercially available statistical program.[h] To compare the findings between the 2 groups, Fisher's exact test or an unpaired Student's *t*-test was carried out as indicated. Repeated measures ANOVA with a post-hoc Dunnett's test was used to evaluate the statistical significance of the differences within the 2 groups with respect to ACTH-stimulated serum cortisol concentrations and laboratory analytes during treatment. A contingency table and McNemar's test were used to determine whether improvements in the clinical signs determined at the serial reassessments were of statistical significance. The data were examined for normality using the Kolmogorov-Smirnov test. $P < .05$ was considered statistically significant. Data represent the mean ± standard deviation (SD).

Results

Clinical Cases and Initial Evaluation

The age range of the dogs in group A was 8–12 years (mean ± SD, 9.7 ± 1.3 years), and their body weight was between 3.44 and 4.90 kg (4.29 ± 0.55 kg). Seven of the 9 dogs were female and neutered; of the 2 male dogs, one was neutered. The breeds were as follows: Maltese (n = 4), Yorkshire Terrier (n = 2), Toy Poodle (n = 1), Shih Tzu (n = 1), and Miniature Schnauzer (n = 1). In group B, the dogs ranged in age from 10 to 14 years (mean ± sd, 11.7 ± 1.7 years), with body

weights ranging from 2.26 to 4.92 kg (4.09 ± 0.89 kg). Three of the 7 dogs were female and neutered; of the 4 male dogs, two were neutered. The breeds were Yorkshire Terrier (n = 3), Shih Tzu (n = 2), Maltese (n = 1), and Pomeranian (n = 1). There were no significant differences between the dogs in groups A and B with respect to age (P = .1017) and weight (P = .8317) at diagnosis.

In the 9 dogs in group A, the clinical signs initially reported by the owners were polyuria/polydipsia (n = 9), polyphagia (n = 8), abdominal distension (n = 9), and dermatologic abnormalities including alopecia (n = 7) (Table 1). Leukocytosis, defined as a total white cell count >1.7 × 10^3 cells/μL, was determined in 5 dogs. All the dogs had ALP activity above the reference range and 55% (5/9) had increased ALT, total cholesterol, triglyceride, and glucose concentrations (Table 2). Urine specific gravity before treatment ranged from 1.005 to 1.032 (1.018 ± 0.003).

In the 7 dogs of group B, the clinical signs were polyuria/polydipsia (n = 7), polyphagia (n = 4), abdominal distension (n = 6), and dermatologic abnormalities including alopecia (n = 5) (Table 1). Four dogs had leukocytosis as defined above. All the dogs had ALP concentrations above the reference range; 42% (3/7) had increased ALT, total cholesterol, and glucose concentrations; and 28% (2/7) had increased triglyceride concentrations (Table 2). Urine specific gravity before treatment ranged from 1.010 to 1.025 (1.017 ± 0.006).

All dogs in groups A and B had abnormal ACTH stimulation test results at enrollment. In group A, the cortisol concentration was 2.35–14.49 μg/dL (10.01 ± 3.80 μg/dL) at baseline and 24.5–41.9 μg/dL (32.65 ± 5.94 μg/dL) at the 1-h post-ACTH stimulation test. In group B, the corresponding values were 2.86–8.83 μg/dL (6.62 ± 2.37 μg/dL) and 25.2–36.9 μg/dL (31.02 ± 4.36 μg/dL), respectively.

In group A, the serum cortisol concentration as determined in the LDDST was 5.17–16.3 μg/dL (11.77 ± 4.26 μg/dL) at baseline, 3.13–7.25 μg/dL (4.88 ± 1.56 μg/dL) after 4 hours of dexamethasone suppression, and 4.11–9.12 μg/dL (6.59 ± 1.77 μg/dL) after 8 hours of dexamethasone suppression. In group B, the corresponding values were 3.69–25.5 μg/dL (9.56 ± 8.02 μg/dL), 2.21–6.78 μg/dL (3.82 ± 1.75 μg/dL), and 2.28–5.83 μg/dL (3.95 ± 1.27 μg/dL), respectively.

The mean left and right adrenal widths were 8.2 ± 2.6 and 7.9 ± 2.7 mm, respectively, in group A and 7.6 ± 2.3 and 7.4 ± 2.0 mm, respectively, in group B. Bilateral symmetrical adrenomegaly was observed in 6 dogs in group A and 4 dogs in group B, as determined by a comparison with the previously reported normal values of adrenal gland size in small-breed dogs.[23] The other dogs in group A (3 dogs) and group B (3 dogs) showed left and right adrenal widths at the upper limits, with each set of adrenal glands being of similar size. On ultrasound, all dogs were found to have an enlarged liver, with a uniformly hyperechogeneic parenchyma.

Evaluation of the Improved Clinical Signs after Trilostane Treatment

In group A, clinical signs, including polyuria and polydipsia, improved in 2 of the 9 dogs after 4 weeks

Table 1. Improvement of the clinical signs during low- (group A) and high-dose (group B) trilostane treatment of 16 dogs with pituitary-dependent hyperadrenocorticism.

		Clinical Signs							
		Water intake		Polyphagia		Alopecia		Pendulous abdomen	
Week	Group	PU/PD[a]	Normal[b]	With	Without	Prominent	Improved	Prominent	Improved
0	A	9	0	8	0	7	0	9	0
	B	7	0	4	0	5	0	6	0
4	A	7	2	7	1	7	0	9	0
	B	3	4	3	1	5	0	6	0
8	A	6	3	6	2	7	0	9	0
	B	2	5	1	3	5	0	6	0
12	A	5	4	6	2	7	0	9	0
	B	2	5	1	3	5	0	5	1
16	A	1	8	4	4	7	0	9	0
	B	0	7	1	3	5	0	5	1
20	A	1	8	4	4	5	2	8	1
	B	0	7	0	4	4	1	5	1
24	A	0	9	0	8	2	5	4	5
	B	0	7	0	4	1	4	1	5

PU, polyuria; PD, polydipsia.

[a]Polyuria and polydipsia were defined as urine output > 50 mL/kg/day and water intake > 100 mL/kg/day, respectively.

[b]Normal water intake was defined as between 40 and 90 mL/kg/day.

All data are expressed as the number of dogs. A contingency table and Fisher's exact test were used for statistical analyses. There was no significant difference between group A (n = 9) and group B (n = 7) after 4, 12, and 24 weeks of treatment.

Table 2. Mean ± standard deviation of laboratory analytes in samples collected from dogs in group A (low-dose treatment) and group B (high-dose treatment).

	Group A (n = 9)				Group B (n = 7)				
Analytes	Week 0	Week 4	Week 12	Week 24	Week 0	Week 4	Week 12	Week 24	Reference Ranges
Na (mmol/L)	148.7 ± 9.9	147.5 ± 4.4	149.9 ± 5.1	148.6 ± 3.6	150.7 ± 5.4	146.8 ± 8.1	146.9 ± 5.4	147.8 ± 3.7	141–152
K (mmol/L)	5.2 ± 0.5	5.3 ± 0.4	5.3 ± 0.5	5.2 ± 0.5	5.2 ± 0.6	5.5 ± 0.6	5.6 ± 0.5	5.3 ± 0.5	4.37–5.35
Na:K ratio	29.0 ± 3.3	27.9 ± 2.2	28.3± 2.1	28.7± 2.8	29.2± 4.0	27.1± 4.0	26.2± 2.6	28.0± 2.7	27–40
ALP (IU/L)	1,824.4 ±1,352.6	1,114.1 ± 921.5	616.1 ± 406.4[a]	313.3 ± 215.4[a]	1,238.0 ± 926.0	256.8 ± 76.3[ab]	255.8 ± 80.2[a]	190.0 ± 53.1[a]	29–97
ALT (IU/L)	127.8 ± 29.8	120.3 ± 20.3	100.7 ± 51.7	66.7 ± 28.3[a]	238.0 ± 111.8	201.3 ± 93.5	146.8 ± 28.9[a]	103.5 ± 52.5[a]	21–102
Cholesterol (mg/dL)	227.6 ± 64.7	191.2 ± 60.6	156.8 ± 64.6	136.0 ± 26.9[a]	227.3 ± 61.0	149.5 ± 13.4[a]	130.8 ± 19.6[a]	111.3 ± 16.1[a]	135–270
Triglyceride (mg/dL)	115.2 ± 22.3	110.0 ± 18.8	104.8 ± 9.0	98.4 ± 11.9	115.3 ± 26.5	97.8 ± 23.4	98.3 ± 18.1	93.3 ± 16.0	21–116
Glucose (mg/dL)	113.8 ± 18.2	107.3 ± 11.0	89.5 ± 10.4[a]	89.5 ± 8.2[a]	117.6 ± 25.1	97.2 ± 15.7	88.8 ± 12.3	94.6 ± 6.1	65–118
Urine specific gravity	1.018 ± 0.003	1.018 ± 0.002	1.022 ± 0.003	1.023 ± 0.003[a]	1.016 ± 0.005	1.024 ± 0.005[a]	1.020 ± 0.005	1.019 ± 0.008	1.015–1.045

Na, sodium; K, potassium; ALP, alkaline phosphatase; ALT, alanine aminotransferase.
[a]Significant difference within group A or group B ($P < .05$).
[b]Significant difference between groups A and B ($P < .05$).

of treatment (Table 1), and in 4 dogs after 12 weeks. By 24 weeks, polyuria and polydipsia had resolved in all of the dogs in this group. Among the 8 dogs with polyphagia, one improved after 4 weeks of treatment, while in all of the affected dogs, it resolved after 24 weeks. By contrast, clinical signs including alopecia and abdominal distension did not begin to improve until after 20 weeks of treatment.

In group B, clinical signs including polydipsia and polyuria improved in 4 of 7 dogs after 4 weeks of treatment. By the end of the study, the improvement in the clinical signs was essentially the same as in group A. Polyphagia improved in 1 of the 4 affected dogs after 4 weeks of treatment, and in all the dogs of this group after 16 weeks. An improvement in the clinical signs including alopecia and abdominal distension followed a similar pattern as in group A, ie, beginning after 20 weeks of treatment and completely resolving in all dogs after 24 weeks. At 20 weeks (at which point we had not planned any reassessments), 2 of 7 dogs in group B presented with clinical signs of vomiting and collapse, with abnormal laboratory findings consistent with hypoadrenocorticism (ACTH-stimulated serum cortisol concentrations were <1.5 μg/dL [1.33 and 1.35 μg/dL, respectively]).

Changes of ACTH-Stimulated Cortisol Concentrations

Significant decreases in ACTH-stimulated serum cortisol concentrations were measured during treatment of the dogs in group A ($P = .013$) and group B ($P = .004$) (Fig 1). In group A, ACTH-stimulated serum cortisol concentrations at 4 weeks were >5.5 μg/dL in all dogs. Sixteen weeks later, 7 of the 9 dogs in group A had ACTH stimulation test results indicative of the control of PDH, with a mean ACTH-stimulated serum

Fig 1. Dot-plots of ACTH-stimulated serum cortisol concentrations in group A and group B dogs treated with low- and high-dose trilostane, respectively. The horizontal bars indicate the mean ± SD. *Mean values were significantly ($P < .05$) lower than those at week 0 (repeated measures ANOVA followed by Dunnett's test). †Significant ($P < .05$) differences between group A (n = 9) and group B (n = 7) at 2, 4, 8, 12, and 16 weeks (unpaired *t*-test).

cortisol concentration of 5.10 ± 1.22 μg/dL (range: 3.12–7.50 μg/dL). The target ACTH-stimulated serum cortisol concentrations were attained in all dogs in group A after 24 weeks of treatment, as indicated by values of 4.17 ± 0.54 μg/dL (range: 3.32–4.86 μg/dL). The downward trend in the ACTH-stimulated serum cortisol concentration continued until the end of the study in every dog.

In group B, the ACTH-stimulated serum cortisol concentration was within the target range in 5 of the 7 dogs after 4 weeks of treatment, with target values achieved in all dogs after 8 weeks of treatment, based on a mean ACTH-stimulated serum cortisol concentration of 3.28 ± 1.13 μg/dL (range of 1.95–5.12 μg/dL). After 8 weeks, the ACTH-stimulated serum cortisol concentrations in 5 dogs were maintained within the target range. As in group A, the target ACTH-stimulated serum cortisol concentration was achieved in all dogs in group B after 24 weeks of treatment.

Before the initiation of trilostane treatment, there was no statistically significant difference in the ACTH-stimulated serum cortisol concentrations between group A and group B. However, significant differences between the 2 groups were determined after 2 (P = .0038), 4 (P = .0001), 8 (P = .0007), 12 (P = .0003), and 16 (P = .0052) weeks of treatment, whereas, as described above, there were no statistically significant differences at 24 weeks (P = .2744).

Adjustment of the Trilostane Dose

In group A, the initial trilostane dose of 0.78 ± 0.26 mg/kg, administered PO every 12 h (1.56 ± 0.53 mg/kg/day) (Fig 2), was maintained for 4 weeks. From then on, the dose was adjusted on the basis of clinical signs and the ACTH-stimulated cortisol concentration to 1.06 ± 0.46 mg/kg every 12 h (2.11 ± 0.93 mg/kg/day) (Table 3). At 16 weeks, a further adjustment was made, to 1.43 ± 0.51 mg/kg every 12 hours (2.86 ± 1.01 mg/kg/day). This dose was maintained as the final dose until the end of the study.

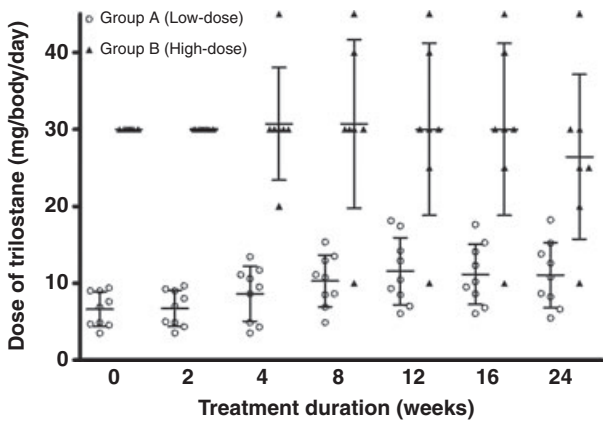

Fig 2. Dot-plots of trilostane doses administered for 24 weeks to dogs with pituitary-dependent hyperadrenocorticism assigned to group A (n = 9) or group B (n = 7). Group A was treated with low-dose trilostane (on a per kg basis) administered PO every 12 h, while group B was treated with high-dose trilostane (on a per-dog basis) administered PO every 24 h. Horizontal bars indicate the mean ± SD.

In group B, the initial trilostane dose was 30 mg/dog, administered PO every 24 hours (7.80 ± 2.50 mg/kg/day) (Fig 2). Four weeks later, the trilostane dose was adjusted on the basis of the clinical signs and the ACTH-stimulated cortisol concentration to 30.71 ± 7.32 mg/dog, administered every 24 hours (7.92 ± 3.01 mg/kg/day). Of note, at 20 weeks, 2 dogs had clinical signs and abnormal laboratory findings consistent with hypoadrenocorticism. In these dogs, trilostane administration was temporarily discontinued, after which their condition improved. At 24 weeks, trilostane was reinstituted with a 50% dose reduction. The final trilostane dose in the 7 dogs of group B was 26.43 ± 10.69 mg/kg, administered every 24 hours (7.07 ± 3.78 mg/kg/day).

Biochemical Analysis and Urinalysis Results

In group A, there were significant decreases in ALP and ALT activities, and total cholesterol and glucose concentrations after the initiation of low-dose trilostane treatment (Table 2). The mean activity of ALP at 12 and 24 weeks was significantly lower than the value before treatment (P = .0009) although it remained above the reference range in all dogs. At 24 weeks, the mean activity of ALT was significantly lower than the value before treatment (P = .0092), and by the end of the study, all 9 dogs had normalized ALT activities. The mean total cholesterol concentration at 24 weeks was significantly lower than the value before treatment (P = .0383). Five of the 9 dogs had normalized total cholesterol concentrations at the end of the study, while in four, they were below the reference range. Serum triglyceride concentrations normalized in all 9 dogs, although the values did not significant differ before and after treatment (P = .5196). The mean blood glucose concentration at 12 and 24 weeks after treatment was significantly lower than the value before treatment (P = .008) and had normalized in all of the dogs by the end of the study. The fluctuations in urine specific gravity were minimal throughout the study, ranging from 1.021 at baseline to 1.028 at the end of the study, at which point the difference was statistically significant (P = .0075). There were no significant changes in sodium and potassium concentrations throughout the study in any of the 9 dogs (P = .8486 and .8687, respectively).

In group B, there were significant decreases in ALP activities and total cholesterol concentrations after the initiation of high-dose trilostane treatment (Table 2). The mean ALP activities at 4, 12, and 24 weeks after treatment were significantly lower than the value before treatment (P = .0026). By contrast, there were no statistically significant differences in ALT activities during the study period (P = .0704). The mean total cholesterol concentrations at 4, 12, and 24 weeks were significantly lower than the value before treatment (P = .0046) and had normalized in all 7 dogs by

Table 3. Change in body weight and trilostane dose during the 24 weeks of the study in groups A and B.

Weeks	0	2	4	8	12	16	24
Group A (n = 9)							
Body weight (kg)	4.29 ± 0.55	4.36 ± 0.57	4.16 ± 0.52	4.00 ± 0.53	4.07 ± 0.59	3.96 ± 0.51	3.88 ± 0.46
Dose (mg/kg/day)	1.56 ± 0.53	1.56 ± 0.53	2.11 ± 0.93	2.61 ± 0.86	2.86 ± 1.01	2.86 ± 1.01	2.86 ± 1.01
Total dose/day	6.67 ± 0.29	6.78 ± 0.30	8.76 ± 0.48	10.45 ± 0.45	11.65 ± 0.59	11.33 ± 0.52	11.09 ± 0.46
No. of dogs in which the dose was increased	0	0	4	5	3	0	0
No. of dogs in which the dose was reduced	0	0	0	0	0	0	0
Group B (n = 7)							
Body weight (kg)	4.09 ± 0.89	4.07 ± 0.94	4.11 ± 0.96	3.96 ± 1.06	3.99 ± 0.91	4.07 ± 0.87	4.00 ± 0.93
Dose (mg/kg/day)	7.80 ± 2.50	7.91 ± 2.70	7.92 ± 3.01	8.25 ± 3.97	7.93 ± 3.76	7.67 ± 3.32	7.07 ± 3.78
Total dose/day	30.00	30.00	30.71 ± 7.32	30.71 ± 10.97	30.00 ± 11.18	30.00 ± 11.18	26.43 ± 10.69
No. of dogs in which the dose was increased	0	0	1	1	0	0	0
No. of dogs in which the dose was reduced	0	0	1	1	1	0	2

the end of the study. Triglyceride values before and after treatment did not significantly differ. Urine specific gravity ranged from 1.005 to 1.025 (1.019 ± 0.008) at the end of the study. After 4 weeks of treatment, the mean urine specific gravity was significantly higher than the value before treatment ($P = .0235$), whereas this was not the case at 12 and 24 weeks. In two of the dogs receiving high-dose trilostane, abnormal serum sodium to potassium ratios of 25:1 and 20:1, consistent with transient hypoadrenocorticism, were measured 20 weeks after treatment.

There were no statistically significant differences in the biochemical analyses and urinalyses between groups A and B. The exception was the mean ALP activity, in which a significant decrease between groups A and B at 4 weeks ($P = .0348$) was determined.

Discussion

The present study supports twice-daily administration of low-dose trilostane as an effective treatment of dogs with PDH. The initial doses given to the 9 dogs treated according to the low-dose protocol ranged from 0.5 to 1.0 mg/kg twice daily (0.78 ± 0.26 mg/kg). These doses are lower than the lowest initial daily dose of 2.2–6.7 mg/kg/day recommended by the drug's manufacturer. In the past, the manufacturer recommended a trilostane dose of 30 mg once daily for dogs weighing less than 5 kg; however, the current trilostane dose recommended for dogs weighing less than 4.5 kg is 10 mg once daily. Among the dogs in the low-dose trilostane group, none required more than 2.0 mg/kg twice daily (1.43 ± 0.51 mg/kg) to control the clinical signs of PDH; however, this should not be taken to mean that this dose is sufficient for all dogs because the group contained a low number of subjects. The mean final dose of 2.86 ± 1.01 mg/kg/day was also much lower than the mean dose of 7.07 ± 3.78 mg/kg/day administered to the high-dose group. The low-dose trilostane protocol used in the management of PDH in this study was similar to that described in other reports evaluating trilostane.[21,22] However, in the present study, the protocol was more time-consuming than the high-dose and frequency protocol recommended by the manufacturer, as one of the treatment objectives was to achieve ACTH-stimulated serum cortisol concentrations within the target range. Nonetheless, this objective was achieved in all dogs after 24 weeks of treatment. On the other hand, in the high-dose group, ACTH-stimulated serum cortisol concentrations within the target range were achieved in all of the dogs as early as after 8 weeks of treatment. Similarly, clinical signs including polydipsia, polyuria, and polyphagia resolved more slowly in dogs treated with low-dose trilostane, perhaps because of the differences in the mean initial trilostane dose given to dogs in the low-dose group versus the high-dose group (1.56 ± 0.53 mg/kg/day divided twice versus 7.80 ± 2.50 mg/kg/day). However, at the end of the study, the improvement in the clinical signs did not differ between the 2 groups. Therefore, despite a lower daily dose, trilostane administered twice daily was very effective in controlling canine PDH. Meanwhile, there are 2 additional disadvantages of giving low-dose trilostane twice daily, apart from the fact that it is more time-consuming than high-dose protocols. Firstly, it is not easy to administer a low dose of trilostane to small dogs because, in most countries, the minimal capsule size is 10 mg. Secondly, once-daily treatment is more client-friendly. With regard to the last point, further studies are needed to determine whether a lower dose given once daily has the same efficacy as a twice-daily dose (for example, a once-daily dose of 2.5 to 3 mg/kg trilostane versus a twice-daily dose of 1.4 mg/kg).

The most serious adverse effect of trilostane is the onset of acute adrenal necrosis. In a dog receiving trilostane for the treatment of HAC, histopathological examination of the adrenal glands evidenced adrenal cortical necrosis with reactive inflammation and fibrosis.[24] Severe adrenal necrosis and persistent isolated hypoadrenocorticism were also observed in a dog with PDH treated with a brief course of trilostane.[20]

Hyperkalemia after 1 week of trilostane treatment was also reported.[13,25] Varying degrees of adrenal necrosis and associated inflammation have been described in postmortems of dogs previously treated with trilostane.[26] It was suggested that the severity of these findings was related to both the dose and the duration of treatment, and that prolonged hypoadrenocorticism was more likely in dogs treated with trilostane for longer than 1 year.[18] In the present study, none of the dogs on the low-dose protocol developed concerning adverse effects; indeed, the major clinical signs of polyuria and polydipsia completely resolved in all cases after 24 weeks of treatment. In addition, none of the treated dogs showed abnormalities suggesting a mineralocorticoid deficiency, such as a decrease in the Na:K ratio or hyperkalemia. Our results are consistent with those of another study evaluating low-dose trilostane treatment in which the drug was shown to be clinically effective and without adverse effects in dogs with PDH.[21,22] However, in two of the dogs receiving high-dose trilostane in our study, clinical signs and abnormal laboratory findings consistent with transient hypoadrenocorticism were observed after 20 weeks of treatment. These findings provide further evidence that the occurrence of adverse effects in dogs treated with trilostane is dose-related.

The ACTH stimulation test is currently recommended for the assessment of HAC control in dogs treated with trilostane.[17,18,25] A recent study concluded that the determination of biochemical analytes, such as ALP and cholesterol, could be useful in this setting.[27] Our study showed that after low- and high-dose trilostane treatment in dogs with PDH, the significant decreases in ALP activity and cholesterol concentration paralleled the reduction in ACTH-stimulated cortisol concentrations. In agreement with this finding, significant reductions in serum ALP activity and cholesterol concentration after control of PDH with trilostane have been reported by other authors.[13,25,27] While the mean ALP activity differed between group A and group B after 4 weeks of treatment, there was no significant difference after 8 weeks. Thus, although the high-dose trilostane protocol yielded a much faster decrease in serum ALP activities than that obtained with low-dose treatment, over the long term (24 weeks), the latter protocol was no less effective. However, the biochemical improvements obtained with the low-dose versus the high-dose protocol must be confirmed in additional long-term assessments.

In the present study, the serum cholesterol concentrations of 3 dogs from group B showed subnormal values after 12 and 24 weeks of treatment. Similarly, another study showed that serum cholesterol concentrations fell below the reference range in 2 dogs treated with trilostane.[25] Improvements in the lipid enzymatic pathway resulting from the decrease in serum cortisol concentrations may account for the reduction in serum cholesterol concentrations after treatment with trilostane,[27] but this effect might not explain the decrease in serum cholesterol concentrations to below reference levels. Further studies on the changes in serum cholesterol concentrations after high-dose trilostane administration are necessary to clarify this point.

In conclusion, the present study supports the use of twice-daily low-dose trilostane administration to dogs with PDH as an effective treatment with the disadvantage being that it is more time-consuming than high-dose protocols. However, because this study involved only a small number of dogs and was limited to those weighing less than 5 kg, a further population-based control study is necessary to confirm these results.

Footnotes

[a]Vetoryl, Arnolds Veterinary Products, Shrewsbury, UK
[b]Hitachi 7020, Hitachi High-Technologies Co, Tokyo, Japan
[c]Humalyte, Human GmbH, Wiesbaden, Germany
[d]Alpha 5, Aloka Co, Tokyo, Japan
[e]Synacthen, Novartis, Basel, Switzerland
[f]Immulite 1000 analyzer, Diagnostic Products Co, Los Angeles, CA
[g]Dexamethasone, Je Il Pharm Co, Daegu, Korea
[h]Prism 5.0, Graph-Pad Software, La Jolla, CA

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