Determination of the concentrations of trilostane and ketotrilostane that inhibit ex vivo canine adrenal gland synthesis of cortisol, corticosterone, and aldosterone

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Objective—To determine whether trilostane or ketotrilostane is more potent in dogs and determine the trilostane and ketotrilostane concentrations that inhibit adrenal gland cortisol, corticosterone, and aldosterone secretion by 50%.

Sample—24 adrenal glands from 18 mixed-breed dogs.

Procedures—Adrenal gland tissues were sliced, placed in tissue culture, and stimulated with 100 pg of ACTH/mL alone or with 5 concentrations of trilostane or ketotrilostane. Trials were performed independently 4 times. In each trial, 6 samples (1 for each time point) were collected for each of the 5 concentrations of trilostane and ketotrilostane tested as well as a single negative control samples. At the end of 0, 1, 2, 3, 5, and 7 hours, tubes were harvested and media and tissue slices were assayed for cortisol, corticosterone, aldosterone, and potassium concentrations. Data were analyzed via pharmacodynamic modeling. One adrenal slice exposed to each concentration of trilostane or ketotrilostane was submitted for histologic examination to assess tissue viability.

Results—Ketotrilostane was 4.9 and 2.4 times as potent in inhibiting cortisol and corticosterone secretion, respectively, as its parent compound trilostane. For trilostane and ketotrilostane, the concentrations that inhibited secretion of cortisol or corticosterone secretion by 50% were 480 and 98.4 ng/mL, respectively, and 95.0 and 39.6 ng/mL, respectively.

Conclusions and Clinical Relevance—Ketotrilostane was more potent than trilostane with respect to inhibition of cortisol and corticosterone secretion. The data should be useful in developing future studies to evaluate in vivo serum concentrations of trilostane and ketotrilostane for efficacy in the treatment of hyperadrenocorticism. (*Am J Vet Res* 2011;72:661–665)

Pituitary-dependent hyperadrenocorticism in dogs has historically been treated most commonly with mitotane, an adrenocorticolytic drug with preferential action on the zona fasciculata and zona reticularis. Much interest has been stimulated in recent years regarding the use of trilostane in the treatment of hyperadrenocorticism in dogs. Trilostane is a competitive inhibitor of 3β-hydroxysteroid dehydrogenase, an essential enzyme in the production pathways of cortisol, corticosterone, aldosterone, androstenedione, and perhaps other enzymes as well.[1] In England, trilostane has been the approved drug of choice for treating hyperadrenocorticism in dogs for the past few years, and it has recently been approved by the FDA for use in the United States.

One of the challenges with successful use of trilostane for the treatment of canine hyperadrenocorticism is monitoring its effectiveness. History and clinical signs are invaluable in assessing a patient's response to treatment (eg, resolution of polyuria, polydipsia, polyphagia, and dermatologic signs). However, an adjuvant biochemical test marker that ensures patient response to treatment and avoidance of hypocortisolism would be advantageous.

Adrenocorticotrophic hormone stimulation testing is the standard for monitoring treatment of hyperadrenocorticism; it provides information regard-

Abbreviations

CI	Confidence interval
CV	Coefficient of variation
EC_{50}	50% effective concentration
E_{max}	Maximum effect

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ing adrenal secretory reserve. However, exogenous ACTH may override the competitive enzyme inhibition that trilostane provides, precluding its ability to indicate effective control.[2] Therefore, identifying a therapeutic serum concentration of trilostane may provide valuable information that could be used in monitoring the efficacy of trilostane in patients with pituitary-dependent hyperadrenocorticism. However, a paucity of published information exists on the pharmacodynamics of trilostane in dogs. Furthermore, the most active metabolite of trilostane, ketotrilostane, is a more potent inhibitor of the target enzyme, compared with trilostane, in some species.[3,4] The relative potency of ketotrilostane versus trilostane and thus the importance of monitoring serum ketotrilostane concentrations in dogs are unknown.

The authors have developed an ex vivo model for assessment of adrenal gland function on the basis of tissue slicing that might provide guidance regarding target concentrations for monitoring purposes. The system offers an advantage over cell culture techniques. Adrenal slices can be maintained short-term and stimulated to secrete hormones, and trilostane administration inhibits cortisol production without any appreciable impairment in adrenal gland viability.[5]

The objectives of the study reported here were to determine the EC_{50} of trilostane and ketotrilostane for adrenal gland secretion of cortisol, corticosterone, and aldosterone and to determine the relative inhibitory potency of trilostane and ketotrilostane for each hormone by use of the ex vivo adrenal gland tissue slice system.

Materials and Methods

Grossly normal adrenal glands were obtained within 10 minutes after healthy dogs used for practice surgeries performed by veterinary students were euthanized. Procedures were performed in accordance with published guidelines and as approved by the Auburn University Institutional Animal Care and Use Committee. The adrenal glands were stored for approximately 12 hours in medium (containing 500 kallikrein inhibitor units of aprotinin[a]/mL and 100 pg of ACTH/mL[b]) at 4°C. On the day of each trial, the glands were placed vertically in an aluminum cylinder and sliced at a 200- to 250-µm thickness with a tissue slicer.[c] Adrenal slices were placed on ice in dishes containing the storage medium. Subsequently, slices were weighed and placed singly on type A titanium mesh roller inserts.[c] The roller inserts were placed into scintillation vials containing Waymouth medium[d] with 500 kallikrein inhibitor units of aprotinin/mL and 100 pg of ACTH/mL[b] at 21°C. After a 30-minute incubation period, the vials were placed in a dynamic organ culture incubator[e] maintained at 37°C in an environment enriched with 95% oxygen and 5% carbon dioxide. Incubation continued under these conditions for 3 hours (T = –3 until T = 0). At T = 0, medium was changed to contain, in addition to ACTH and aprotinin, either methanol (negative control) or trilostane or ketotrilostane. The drug concentrations chosen inhibit steroid hormone synthesis in guinea pig adrenal gland tissue[4] and rat ovarian tissue[3] and encompass peak serum trilostane concentrations obtained after oral administration of trilostane to dogs.[e] Concentrations tested for trilostane were 140, 700, 1,400, 4,200, and 7,000 ng/mL, and for ketotrilostane were 40, 200, 400, 1,200, and 2,000 ng/mL. Incubation continued after the addition of the drug for an additional 4 hours (T = 0 through T = 4). Samples were harvested at T = –3, –2, –1, 0, 2, and 4. Thus, for each compound in each trial, samples were collected at each of 6 time points for each of the 5 concentrations of trilostane and ketotrilostane tested as well as a single negative control. Two medium samples were collected at T = –2, –1, 0, 2, and 4 (ie, a total of 50 vials each for trilostane and ketotrilostane and 10 vials for the negative control, for a total of 110 medium samples/trial). Tissue slices were collected at T = –3, 0, and 4. Trials were performed independently 4 times. All slices treated with a single drug and concentration came from a single adrenal gland. All medium samples and tissue slices collected were divided for hormonal assays (cortisol, corticosterone, and aldosterone; medium samples were not collected at T = –3), cytosolic potassium concentrations, and histologic examination.

Medium samples from both tubes per concentration were assayed for cortisol[f] and aldosterone[g] by use of validated assays.[6,7] The corticosterone assay[h] was performed per manufacturer's instructions. Four samples were randomly chosen and diluted 1:2, 1:4, and 1:8 in the diluent supplied with the kit and assayed. Mean percentage recoveries for the 1:2, 1:4, and 1:8 dilutions were 83.4%, 98.1%, and 83.1%, respectively. The slopes of the lines obtained from the dilutional studies were similar (*t* test; $P > 0.05$) to those from the respective standard curves. Per the manufacturer, intra-assay CVs for samples of high, medium, and low concentration were 4.4%, 7.1%, and 10.3%, respectively, whereas the interassay CVs were 6.5%, 7.2%, and 7.1%, respectively. Cross-reactivities for cortisol and aldosterone were 0.05% and 0.03%, respectively (per the manufacturer). Sensitivity of the assay was 25 ng/mL. All samples were assayed in duplicate.

Cytosolic potassium concentrations were measured to assess adrenal gland slice viability.[8] Homogenates of 1 slice/drug concentration were collected at all time points and assayed for cytosolic potassium concentration (ie, a total of 30 slices each for trilostane and ketotrilostane and 6 slices for the negative control, for a total of 66 slices/trial). To prepare the homogenates, adrenal gland slices were homogenized in 1.0 mL of distilled water at approximately 20,000 cycles/min for 30 to 45 seconds until adequately homogenized. Homogenates were stored at –80°C until assays could be performed. Potassium assays were performed within 12 weeks of collection via a commercial electrolyte analyzer[i] after thawing and vortexing samples.

One adrenal gland slice exposed to each concentration of trilostane or ketotrilostane was submitted for histologic examination. Adrenal slices were placed in neutral-buffered 10% formalin, sectioned transversely, and embedded in paraffin with the cut surface facedown, allowing sectioning across the greatest diameter of the tissue slice. Tissues were sectioned with a microtome at 5 µm, stained with H&E, and examined histologically by 2 of the authors (EMW and ALM). Tissues were evaluated and assigned a score on the basis of the following features: cell membrane (3+ = very crisp

margins, 2+ = moderately crisp margins, and 1+ = indistinct margins), cytoplasm (3+ = very distinct vacuoles, 2+ = moderately distinct vacuoles, and 1+ = indistinct vacuoles), and nuclear morphology (3+ = approx 80% to 100% dispersed chromatin and no evidence of apoptosis, 2+ = approx 50% to 80% dispersed chromatin and minimal apoptosis, and 1+ = many apoptotic nuclei). Histologic scores for adrenal glands were totaled. Possible scores ranged from 3 (poorest) to 9 (best).

Statistical analysis—Results are reported as mean ± SD values. For all time points at which 2 medium samples were collected (ie, T = –2, –1, 0, 2, and 4), the CV was calculated. The mean sample pair CV was calculated for each of the hormones.

Percentage inhibition was calculated as the percentage difference between the T = 0 (maximal stimulation) and T = 4 (maximal inhibition) time points divided by the weight of the slice to correct for differences in slice size. To detect differences in percentage inhibition among drug concentrations, data were analyzed by use of a 1-way ANOVA with a commercial statistical program.[j] If a significant difference was detected, post hoc comparisons were made by use of the Student-Newman-Keuls method (all-pairwise) and multiple comparison test.

The relationship between drug or metabolite concentrations and effect was assumed to be direct. The response-effect was subjected to pharmacodynamic modeling[k] that determined the E_{max} and EC_{50} after removal of a baseline effect parameter. The best model was chosen on the basis of the goodness of fit of the estimated statistical model by use of Akaike information criterion.

For comparison of total morphological score over time and among treatments, a 1-way ANOVA was used. For all statistical analyses, values of $P \leq 0.05$ were considered significant.

Results

For cortisol, corticosterone, and aldosterone, the mean sample pair CVs were 20%, 20%, and 35%, respectively. The CV for aldosterone data was judged to be unacceptable, and the data were not further analyzed.

Concentrations of cortisol and corticosterone progressively increased until T = 0, reflecting adequate recovery of the tissue slices from slicing (**Figure 1**). The decrease between T = 0 and T = 2 reflected the medium change; the concentrations at T = 4 indicated the effect of the drugs on hormone secretion, with the 5 concentrations of trilostane and ketotrilostane generally correlating with an increased degree of inhibition (Figures 1 and 2).

With respect to cortisol, percentage inhibition of the 4 higher concentrations of trilostane was significantly ($P = 0.001$) greater, compared with results for the negative control (**Table 1**). In addition, the 3 highest trilostane concentrations inhibited cortisol secretion significantly more than did the lowest concentration. With respect to ketotrilostane, the 4 higher concentrations caused significantly ($P = 0.002$) greater inhibition of cortisol secretion than did the negative control.

With respect to corticosterone, percentage inhibition attributable to the 4 higher concentrations of trilostane was significantly ($P = 0.001$) greater, compared with results for the negative control (Table 1). All 5 concentrations of ketotrilostane significantly ($P < 0.001$) inhibited corticosterone secretion, compared with results for the negative control.

Pharmacodynamic modeling of trilostane and ketotrilostane effects on cortisol secretion identified an E_{max} of percentage inhibition of 80.4% (90% CI, 69.2% to 91.5%) and 68.6% (90% CI, 11.3% to 125.9%), respectively, and an EC_{50} of 480 ng/mL (90% CI, 142.3 to 817.7 ng/mL) and 98.4 ng/mL (90% CI, 38.9 to 157.9 ng/mL), respectively. Thus, ketotrilostane was 4.9 times as potent in inhibiting cortisol secretion as the parent compound trilostane. Additionally, pharmacodynamic modeling of trilostane and ketotrilostane effects on corticosterone secretion resulted in an E_{max} of percentage inhibition of 81.4% (90% CI, 76.9% to 85.9%) and 78.5% (90% CI, 72.8% to 84.2%) and an EC_{50} of 95.0 ng/mL (90% CI, 30.3 to 159.6 ng/mL) and 39.6 ng/mL (90% CI, 22.3 to 56.9 ng/mL), respectively. Thus, ketotrilostane was 2.4 times as potent in inhibiting corticosterone secretion as trilostane.

Cytosolic potassium concentrations decreased over the first hour of incubation and then remained stable throughout the remainder of the study (data not

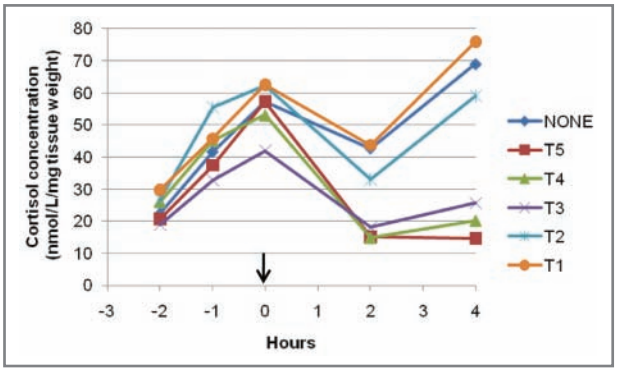

Figure 1—Secretion of cortisol by canine adrenal gland slices in response to inhibition by 5 concentrations of trilostane (T1 = 140 ng/mL, T2 = 700 ng/mL, T3 = 1,400 ng/mL, T4 = 4,200 ng/mL, and T5 = 7,000 ng/mL) or a negative control (NONE). Arrow denotes medium change and addition of methanol (negative control), trilostane, or ketotrilostane. Data are means of 4 trials.

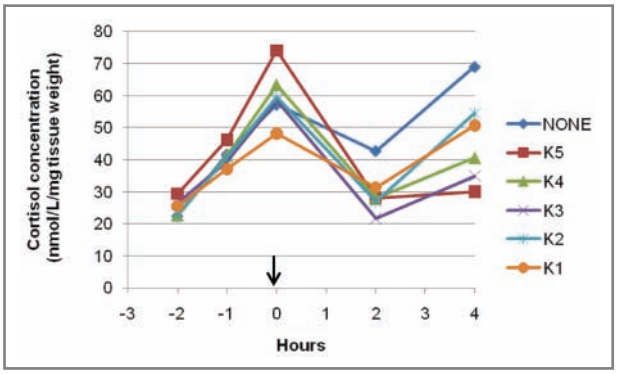

Figure 2—Secretion of cortisol by canine adrenal gland slices in response to inhibition by 5 concentrations of ketotrilostane (K1 = 40 ng/mL, K2 = 200 ng/mL, K3 = 400 ng/mL, K4 = 1,200 ng/mL, and K5 = 2,000 ng/mL) or a negative control (NONE). *See* Figure 1 for remainder of key.

Table 1—Mean ± SD percentage inhibition of cortisol and corticosterone concentrations after 4-hour exposure of adrenal gland slices to 5 concentrations of trilostane (T1 to T5) and ketotrilostane (K1 to K5).

Drug concentration	Cortisol	Corticosterone
Trilostane		
None	−15.3 ± 36.5	30.5 ± 17.5
140 ng/mL (T1)	7.8 ± 3.5	54.7 ± 16.5
700 ng/ml (T2)	49.7 ± 13.4[a]	64.2 ± 20.9[a]
1,400 ng/mL (T3)	56.0 ± 6.3[a,b]	70.0 ± 11.6[a]
4,200 ng/mL (T4)	72.5 ± 14.1[a,b]	85.5 ± 5.0[a]
7,000 ng/mL (T5)	76.2 ± 5.6[a,b]	85.4 ± 1.3[a]
Ketotrilostane		
None	−15.3 ± 36.5	30.5 ± 17.5
40 ng/mL (K1)	35.4 ± 29.5	69.5 ± 10.6[a]
200 ng/mL (K2)	50.4 ± 19.5[a]	69.7 ± 11.6[a]
400 ng/mL (K3)	62.5 ± 9.3[a]	74.1 ± 10.7[a]
1,200 ng/mL (K4)	55.6 ± 27.2[a]	73.4 ± 19.5[a]
2,000 ng/mL (K5)	61.7 ± 10.0[a]	79.5 ± 6.9[a]

[a]Significantly ($P < 0.05$) greater than the value for the negative control (None). [b]Significantly ($P < 0.05$) greater than the value for T1.

shown), indicating recovery and viability of the adrenal gland slices.[8] No lesions were observed in any adrenal gland during gross examination before or during tissue slicing or in any section examined histologically. No cytomorphological changes attributable to disease were observed in histologic sections. Total morphology scores from histologic evaluation of incubated tissue sections were not significantly ($P = 0.37$) different over time. In addition, total morphology scores were not significantly ($P = 0.340$) different among treatments.

Discussion

The application of an ex vivo slice technique with tissue organ culture as a means of assessing adrenal gland function and the effects of trilostane has been proven to be a viable technique.[5] To our knowledge, no other researchers have applied the technique to the study of canine adrenal glands. Our efforts to determine relevant pharmacodynamic information regarding trilostane, a drug recently approved by the FDA for the treatment of hyperadrenocorticism in dogs, as well as its primary metabolite ketotrilostane may serve as a foundation for further in vivo studies of the drug. Different protocols for monitoring the efficacy of trilostane have been proposed, and all involve the use of ACTH stimulation testing to evaluate adrenal reserve. An alternative means for monitoring may be evaluation of serum trilostane concentration, ketotrilostane concentration, or both, similar to evaluation of phenobarbital therapy, for example.

Ketotrilostane is more potent than trilostane in rats and guinea pigs,[3,4] but whether the same is true for dogs has not been previously evaluated, to our knowledge. Interestingly, in the present study, ketotrilostane was 4.9 times as potent in inhibiting cortisol synthesis, compared with its parent compound trilostane. Ketotrilostane in guinea pigs and rats, in comparison, is 2.4 and 1.7 times as potent as trilostane, respectively.[6,11] Thus, if serum drug concentration monitoring is proven to be useful with trilostane, it may be necessary to measure serum ketotrilostane concentrations in conjunction with or in place of trilostane.

The reason why the relative potency of ketotrilostane is higher in dogs is not apparent. Ketotrilostane's affinity for enzyme active sites or for enzymes other than 3β-hydroxysteroid dehydrogenase involved in cortisol synthesis may be much higher than that of trilostane. Additionally, although 3β-hydroxysteroid dehydrogenase is the primary adrenal enzyme inhibited in several species,[3,9–11] trilostane in dogs may have competitive inhibitory effects on other enzymes involved in adrenal gland steroid hormone synthesis such as 11β-hydroxysteroid dehydrogenase[1]; the affinity of ketotrilostane for the alternative enzymes may be greater than that of trilostane. Lastly, although trilostane's mechanism of action is that of enzymatic blockade, and given that a previous human study[12] revealed that trilostane does not react with hormonal sex steroid receptors, the possibility that ketotrilostane may interfere with cortisol production more effectively than trilostane in canine adrenal glands via other mechanisms cannot be definitively excluded.

Rationale behind the selection of drug concentrations used in the study was based on pharmacological data obtained after treating dogs ranging from 8.2 to 11.4 kg in body weight with 30 mg (approx 3 mg/kg) of trilostane twice daily for 5 days.[e] The middle concentrations used in the present study (1,400 and 400 ng/mL for trilostane and ketotrilostane, respectively) were the mean maximal concentrations obtained. Concentrations of trilostane and ketotrilostane were selected to bracket these concentrations (ie, the lowest concentrations used represented 10% of the middle, and the highest concentration used represented 5.0 times the middle). The EC_{50} for ketotrilostane with regard to cortisol secretion was 98.4 ng/mL, a concentration in the lowest 5% used in the study (range, 40 to 2,000 ng/mL) and thus perhaps not highly accurate. Future studies are needed that use lower concentrations to further define the EC_{50} of ketotrilostane.

The ramifications of the histologic findings as pertains to the effects of drug exposure are difficult to determine given that adrenal slices were exposed to each drug for only 4 hours. Necrosis secondary to trilostane administration is a potential adverse effect of treatment, but the changes likely require at least a few weeks of drug administration, and the necrosis may be caused by high endogenous ACTH concentrations and not the trilostane itself.[13,14] Harvesting after dogs were euthanized, storage overnight, and slicing may have contributed to the observed morphological changes. In light of this, the finding that morphological scores were not significantly different among time points or between slices treated with trilostane and ketotrilostane versus the negative control likely indicates that inhibitory effects of trilostane and ketotrilostane were chiefly attributable to drug exposure and not to concomitant tissue degradation.

Acknowledged limitations of this study include, first, the constraint encountered when performing ex vivo rather than in vivo testing. The latter method may more accurately depict pharmacodynamic data in a patient; however, the information obtained here may be used as a starting point in the design of future studies. Second, drug concentrations used in this study were

much higher than obtained EC_{50} values, especially in the case of ketotrilostane. As stated, the concentrations were empirically selected on the basis of pharmacokinetic data obtained from the drug manufacturer. Given our data, a lower, narrower range of drug concentrations should be used for future studies. Lastly, in this study, the concentration of ACTH used was at the upper limit of the reference range; the concentration was chosen to simulate normal physiologic conditions in the ex vivo model and was proven in an initial study[5] to adequately stimulate adrenal gland secretion. However, endogenous ACTH concentrations in patients treated with trilostane are substantially greater than pretreatment concentrations and greater than normal physiologic concentrations.[1,15] One study[15] revealed that endogenous ACTH concentrations were significantly different between pretreatment- and posttreatment time points, with median pretreatment values of 102 pg/mL and posttreatment values of 246 pg/mL. Increased concentrations of ACTH could cause increased adrenal gland stimulation[2] that may theoretically override trilostane's competitive inhibitory effects. Future studies could be performed with different ACTH concentrations to further evaluate the effect of concentration.

It was disappointing that the aldosterone data were not more informative. Given the high CV (35%), the variability between slices was deemed to be unacceptably high and the data were not further evaluated. A possible reason for the greater CV in the aldosterone data, compared with that calculated for cortisol and corticosterone, is that the zona glomerulosa layer, the outermost layer of the cortex, may have been more susceptible to damage during the harvesting or slicing portions of the study.

Use of the ex vivo adrenal gland slice model to evaluate relative potencies of trilostane and ketotrilostane in inhibiting adrenocortical secretion of cortisol and corticosterone served as a useful technique in arriving at preliminary pharmacodynamic data. The data should be useful for future studies that evaluate in vivo serum concentrations of trilostane and ketotrilostane in further evaluating efficacy in the treatment of hyperadrenocorticism. Because of the greater potency of ketotrilostane, compared with trilostane, serum ketotrilostane concentrations must be measured to evaluate in vivo pharmacodynamics. In addition, because the EC_{50} and E_{max} may differ between hormones, the effect of trilostane and ketotrilostane should be evaluated for each hormone individually. The inhibition of cortisol secretion reflects efficacy, whereas the inhibition of aldosterone and possibly corticosterone secretion suggests toxicity and undesired adverse effects.

a. USB Corp, Cleveland, Ohio.
b. Cortrosyn, Amphastar Pharmaceuticals Inc, Rancho Cucamonga, Calif.
c. Brendel/Vitron, Vitron Inc, Tucson, Ariz.
d. Sigma-Aldrich, Waymouth MB 752/1 Medium, St Louis, Mo.
e. Torr E, Dechra Pharmaceuticals, Shrewsbury, Shropshire, England: Personal communication, 2005.
f. Coat-a-Count cortisol assay, Siemens Healthcare Diagnostics, Deerfield, Ill.
g. Coat-a-Count aldosterone assay, Siemens Healthcare Diagnostics, Deerfield, Ill.
h. ImmuChem Double Antibody Corticosterone 125I Kit, MP Biomedicals LLC, Orangeburg, NY.
i. Medica EasyLyte, Bedford, Mass.
j. SigmaStat for Windows, version 1.0, Jandel Scientific SPSS Inc, Chicago, Ill.
k. WinNonlin Professional, version 4.1, Pharsight Corp, Mountain View, Calif.

References

1. Sieber-Ruckstuhl NS, Boretti FS, Wenger M, et al. Cortisol, aldosterone, cortisol precursor, androgen and endogenous ACTH concentrations in dogs with pituitary-dependent hyperadrenocorticism treated with trilostane. *Domest Anim Endocrinol* 2006;31:63–75.
2. Neiger R, Ramsey IK, O'Conner J, et al. Trilostane treatment of 78 dogs with pituitary-dependent hyperadrenocorticism. *Vet Rec* 2002;150:799–804.
3. Robinson DT, Earnshaw RJ, Mitchell R. The bioavailability and metabolism of trilostane in normal subjects, a comparative study using high pressure liquid chromatographic and quantitative cytochemical assays. *J Steroid Biochem* 1984;21:601–605.
4. Lambert A, Mitchell R, Frost J, et al. A simple in vitro approach to the estimation of the biopotency of drugs affecting adrenal steroidogenesis. *J Steroid Biochem* 1985;23:235–238.
5. Behrend EN, Boothe DM, Tillson DM, et al. Creation of a canine adrenal gland slice model for ex vivo study of adrenocortical function. *J Vet Intern Med* 2007;21:645.
6. Kemppainen RJ, Thompson FN, Lorenz MD. Use of a low dose synthetic ACTH challenge test in normal and prednisone-treated dogs. *Res Vet Sci* 1983;35:240–242.
7. Behrend EN, Weigand CM, Whitley EM, et al. Corticosterone- and aldosterone-secreting adrenocortical tumor in a dog. *J Am Vet Med Assoc* 2005;226:1662–1666.
8. Fisher RL, Ulreich JB, Nakazato PZ, et al. Histological and biochemical evaluation of precision-cut liver slices. *Toxicol Methods* 2001;11:59–79.
9. Jungmann E, Magnet W, Rottman-Kuhnke U et al. The inhibiting effect of trilostane on adrenal steroid synthesis: hormonal and morphological alterations induced by subchronic triolostane treatment in normal rats. *Res Exp Med (Berl)* 1982;180:193–200.
10. McGee JP, Shaw PN. The pharmacokinetics of trilostane and ketotrilostane in an interconverting system in the rat. *Pharm Res* 1992;9:464–468.
11. Lambert A, Frost J, Mitchell R, et al. On the assessment of the in vitro biopotency and site(s) of action of drugs affecting adrenal steroidgenesis. *Ann Clin Biochem* 1986;23:225–229.
12. Tueni E, Devleeschouwer N, Leclercq G, et al. Endocrine effects of trilostane: in vitro and in vivo studies. *Eur J Cancer Clin Oncol* 1982;23:1461–1467.
13. Chapman PS, Kelly DF, Archer J, et al. Adrenal necrosis in a dog receiving trilostane for the treatment of hyperadrenocorticism. *J Small Anim Pract* 2004;45:307–310.
14. Reusch CE, Sieber-Ruckstuhl N, Wenger M, et al. Histological evaluation of the adrenal glands of seven dogs with hyperadrenocorticism treated with trilostane. *Vet Rec* 2007;160:219–224.
15. Witt A, Neiger R. Adrenocorticotropic hormone levels in dogs with pituitary-dependent hyperadrenocorticism following trilostane therapy. *Vet Rec* 2004;154:399–400.