

STANDARD ARTICLE

Evaluation of compounded trilostane packets for dogs with naturally occurring hyperadrenocorticism

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Abstract

Background: Dogs treated for naturally occurring hyperadrenocorticism (NOH) in Korea often appear to require higher doses of trilostane than recommended by authors in the United States, Europe, or the United Kingdom. This phenomenon may be related to compounding trilostane into packets, which is a common practice among veterinary clinics in Korea.

Objective: Analyze packets filled by hand and others filled using a semi-automatic packing device for accuracy of trilostane strength.

Animals: Medication packets prepared for 3 dogs with preexisting prescriptions for NOH were analyzed.

Method: A trilostane assay was developed for analysis. Trilostane (Vetoryl) capsules were used as clinical controls. Forty-four medication packets containing trilostane (Vetoryl), prepared by 3 clinicians for 3 dogs with NOH were analyzed.

Results: Of 44 trilostane-containing packets, only 40.9% (18 packets) had acceptable strength of trilostane.

Conclusions and Clinical Importance: Clinicians should be aware that compounding trilostane into packets fails to consistently provide measured amounts of trilostane, potentially interfering with response to treatment for NOH in dogs.

KEYWORDS

compound, Cushing's, dog, drugs, hyperadrenocorticism

1 | INTRODUCTION

When first introduced to veterinary medicine as a drug that interferes with cortisol synthesis, the manufacturer-recommended once daily PO dosage of trilostane (Vetoryl, Arnolds Veterinary Products Ltd, Shropshire, UK) was approximately 3 to 10 mg/kg for

treatment of dogs with naturally occurring hyperadrenocorticism (NOH).¹⁻³ Adverse reactions associated with glucocorticoid or mineralocorticoid deficiency or both were reported in some of the trilostane-treated dogs.²⁻⁹ Subsequent studies determined that lower trilostane doses, given twice daily, markedly decreased the frequency of adverse effects while maintaining or improving efficacy in controlling clinical signs of NOH.^{8,10,11} The starting dosage of US Food and Drug Administration (FDA) approved trilostane formulation is 2.2 to 6.7 mg/kg PO q24h (Vetoryl insert). However, most authors currently

Abbreviations: DMSO, dimethyl sulfoxide; NOH, naturally occurring hyperadrenocorticism; RSD, relative SD.

Edward C. Feldman and Kyoung-won Seo contributed equally to this study.

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recommend initial trilostane dosages of approximately 1 mg/kg PO q12h and final doses often plateau at 0.5 to 2.0 mg/kg.^{10,12,13}

Although no published study is available, anecdotally, many trilostane-treated dogs with NOH in Korea require higher doses to control the condition than those currently reported by authors from the United States, Europe, or the United Kingdom. This discrepancy in dosage requirement can be challenging for veterinarians and owners. One explanation for dose variance might be the customary method of administering PO medications to dogs in Asia. Small animal veterinarians in Korea commonly create packets, which are produced by combining and pulverizing a mixture of all prescribed drugs into a homogenous powder to be administered as is or sprinkled on the pet's food by the owner one or more times daily. Our purpose was to measure trilostane strength in a sample of packets to compare with the amount believed to be in the powder.

2 | MATERIALS AND METHODS

2.1 | Trilostane assay

A trilostane assay was developed using reagents and chemicals of high-performance liquid chromatography (HPLC) analytical grade. An Agilent 1260 Infinity HPLC system (Santa Clara, California) equipped with a pump (model G7111A), an auto sampler (model G7129A), Kinetex C18 (150 × 4.6 mm, 5 µm particle size) column (Torrance, California), and ultraviolet detector (model G4260B) operated at 254 nm was used for the trilostane assay and its validation. The mobile phase utilized a gradient elution of 10% distilled water (solvent A) and 90% acetonitrile (solvent B) for 9 minutes. Flow rate was 0.8 mL/min with an injection volume of 8 µL, a running time of 13 minutes, and an operating temperature of 30°C. Data collection and processing were performed using openLAB CDS software (ChemStation Edition, Germany).

Standard solutions were prepared by dissolving trilostane obtained from Sigma-Aldrich (St. Louis, CAS number: 13647-35-3) in dimethyl sulfoxide (DMSO). Serial dilutions then were created (100, 50, 25, 12.5, and 6.5 µg/mL). Validation was based on specificity, linearity, range, accuracy, and precision (Guidelines GL1 (definition and terminology) and GL2 (methodology) of the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products). Specificity of the method was evaluated by injecting 8 µL solutions of blank, standard, sample, and placebo separately. Linearity was assessed using 3 injections of the 5 different standard concentration solutions. A calibration curve was developed by plotting the average of 3 peak areas against concentrations. A linear equation was derived by regression analysis. Three injections of standard trilostane solutions at 5 different concentrations were analyzed. Percent accuracy was calculated using the formula: (% accuracy = [average of actual concentrations/injected concentration] × 100). Intraassay precision was determined using 3 injections of standard trilostane solutions at the 5 different concentrations. Interassay precision was determined by analyzing 3 injections of the standard trilostane solutions on 3 different days. The precision of the method was represented as the relative SD (RSD).

2.2 | Samples assayed

Commercially available trilostane (Vetoryl, Dechra Pharmaceuticals, North Yorkshire, UK) 60 mg capsules were used as clinical controls. The content of each capsule was dissolved individually in DMSO and centrifuged at 1350 rpm for 5 minutes. Each supernatant solution was diluted with methanol to achieve concentrations of 50, 25, and 12.5 µg/mL and analyzed for trilostane strength. Forty-four medication packets containing trilostane (Vetoryl), prepared by 3 clinicians for 3 dogs with preexisting prescriptions for NOH were analyzed. The 44 packets included 16 claimed to have 5.9 mg of trilostane each (also pimobendan, enalapril, and torsemide), 14 packets claimed to have 11.3 mg trilostane each (also clopidogrel, silymarin, ursodeoxycholic acid, and enalapril), and 14 packets claimed to have 11.3 mg of trilostane each (also telmisartan, silymarin, amlodipine, and clopidogrel). Each clinician prepared packets routinely; 30 prepared by hand using a medical grade micro-spatula and 14 prepared using a semi-automatic drug packing device. One packet containing no trilostane but all other medications (placebo) also was prepared by each clinician. The content of all 47 packets was dissolved individually in DMSO and centrifuged at 1350 rpm for 5 minutes. The supernatant was diluted with methanol to achieve concentrations of 37.5 and 29.4 µg/mL before being analyzed. These concentrations were within the assay's optimal range of 10 to 50 µg/mL. Trilostane strength was categorized as either acceptable or unacceptable using standards of the US Pharmacopeia General Chapter on Pharmaceutical Compounding (Chapters 795 and 797), which states a general acceptance criterion for compounded products to be ±10% of the strength stated on the label.

3 | RESULTS

3.1 | Trilostane assay

Chromatogram results showed no interfering peaks at the retention time of trilostane (average retention time, 7.698 ± 0.005), which confirms the purity of the trilostane peak as well as the specificity of the developed method. The linearity of the calibration graph was assessed by analyzing 5 different standard trilostane concentrations that ranged from 6.25 to 100 µg/mL. Regression analysis confirmed a linear relationship between the concentration of trilostane and the area under the peak, with a linear equation of $y = 11.32216x - 2.70694$ and a determination coefficient value (R^2) of 0.99994. Intraassay accuracy values were 99.0% to 103.6%, and precision RSD values were 0.23% to 4.6% at the 5 concentrations of standard trilostane solutions. Interassay accuracy values and precision RSD values were 98.9% to 105.9% and 1.2% to 4.7%, respectively.

3.2 | Analysis of commercial trilostane (Vetoryl)

Four capsules were each analyzed in triplicate at 3 concentrations: 12.5, 25, and 50. Average accuracy values at these concentrations

were 101.0%, 102.5%, and 101.6%, respectively. Average precision values at concentrations 12.5, 25, and 50 µg/mL were 2.0%, 1.7%, and 1.5%, respectively.

3.3 | Analysis of medication packets

Trilostane was not detected in any of the placebo packets. Of the 44 packets containing trilostane, 40.9% (18 packets) met acceptable standards for trilostane strength and 59.1% (26 packets) did not (Table S1). No significant difference in accuracy results were found between packets prepared by hand and those prepared using a packing device.

4 | DISCUSSION

Compounding >1 medication into a powder placed in packets is a common practice among Korean veterinarians. The resulting powder is suggested to be given with water or sprinkled on the pet's food. The efficacy of this practice has not been assessed. Our results indicate that serious inaccuracies can occur when dispensing trilostane in packets. Dogs given packet medications may receive inaccurate doses approximately 59.1% of the time, based on our results. These dogs would appear to require higher doses of trilostane than dogs in other countries. Furthermore, participating clinicians knew that some packets were to be assayed for trilostane strength and they may have been more careful than the average person performing this task. More variability of drug strength in packets likely would have been identified if packets prepared by a larger number of individuals had been assessed.

Compounded packets usually are prepared using 1 of 2 methods, by hand or using a semi-automatic drug-packing device. When done by hand, equal amounts of medications are carefully divided among packets using a micro-spatula. When using semi-automatic drug packing devices, medications are inserted into the device which then distributes the contents into an assigned number of packets. An extra step of leveling out medications may be needed, depending on the device. Although it may be portrayed as an advantage to prepare packets using a semi-automatic drug packing device, our results suggest that this method still is not adequate. Dogs chronically given packets of medication containing less than the anticipated quantities of trilostane may be common in dogs in Korea thought to require higher doses than suggested by the veterinary literature.

Delivering medication using packets is probably only a partial answer to the question of trilostane dose requirements in Korean dogs. One of the remaining factors is the use of licensed trilostane (Vetoryl). Trilostane strength from nonlicensed sources has been shown to be inconsistent, but alternative sources often are used for prescribed medications.¹⁴ Our study differs from a previous study in that all samples that were analyzed used FDA-approved products as a source of trilostane. As a result, claimed trilostane strengths can be associated exclusively with the compounding methods used.

Despite production of 5 mg and 10 mg Vetoryl capsules, these doses still are not small enough for use in many small dogs, especially

considering the recent recommendations of an initial dosage of 0.2 to 1 mg/kg PO q12h.^{11,15} It is even more difficult to make dose adjustments using only licensed products. Production of smaller doses of validated licensed capsules would be ideal. However, if the only available option is unvalidated compounded trilostane, clinicians should keep in mind that this practice can lead to variations in trilostane concentrations, resulting in inadequate control of NOH.

5 | CONCLUSION

Analysis of 44 packets of compound trilostane showed that only 40.9% (18 packets) met acceptable standards for trilostane strength. Clinicians should be aware that compounding trilostane into packets can result in variations in concentration, potentially hindering effective treatment of dogs with NOH.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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