The Veterinary Journal 194 (2012) 349–353

Contents lists available at SciVerse ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Endocrine effects of inhaled budesonide compared with inhaled fluticasone propionate and oral prednisolone in healthy Beagle dogs

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ARTICLE INFO

Article history:
Accepted 25 April 2012

Keywords:
Adrenal suppression
Inhaled corticosteroids
Dog
Oral corticosteroid
ACTH stimulation test

ABSTRACT

Orally administered corticosteroids are commonly used to treat chronic respiratory disease, but adverse effects suggest that the inhalation route may be safer. To compare the systemic effects of inhaled and oral corticosteroids, a prospective, randomised, placebo-controlled cross-over study was conducted. Six healthy neutered female Beagle dogs were randomly allocated to four treatment groups: (1) budesonide inhalation (200 μg twice daily); (2) fluticasone inhalation (250 μg twice daily); (3) oral prednisolone (1 mg/kg once daily); and (4) placebo inhalation (room air twice daily). Each treatment and wash-out period lasted 4 weeks. The endocrine status of each dog was assessed on days 0, 28 and 35 using the adrenocorticotropic hormone (ACTH) stimulation test. The effects of treatments were assessed using a linear mixed effects model.

After the 4 week treatment period, a significant decrease was observed in the basal serum cortisol level of the prednisolone group ($P < 0.03$), and a decrease was also seen in the ACTH-stimulated peak cortisol levels of both the prednisolone and fluticasone groups ($P < 0.001$), compared with the budesonide group in which no suppression was detected. The results showed that cortisol production in dogs was strongly suppressed by oral prednisolone and by inhaled fluticasone.

Introduction

Chronic inflammatory airway diseases, most notably chronic bronchitis and eosinophilic bronchopneumopathy, are common causes of coughing in dogs. Cornerstone therapies have relied on the oral administration of corticosteroids, although this is frequently associated with undesirable adverse effects. Inhaled corticosteroids (ICSs) have replaced oral products as the first-line anti-inflammatory therapy for asthma in humans, and species-specific modification of inhalation chambers and masks has led to ICSs being more commonly used in companion animals. However, to our knowledge, there is only a single report describing the therapeutic use of fluticasone propionate (FP) and beclomethasone dipropionate in canine chronic inflammatory airway disease (Bexfield et al., 2006). No studies comparing the clinical efficacy of different ICSs in dogs have been published.

The most common systemic adverse effects related to oral corticosteroid treatment in dogs are polydipsia and polyuria, polyphagia, weight gain, hair loss and suppression of the hypothalamic–pituitary–adrenal (HPA) axis, leading to decreased endogenous cortisol production. ICSs are also capable of causing dose-related systemic adverse effects, but to a lesser extent than orally administered preparations (Ahmet et al., 2011). ICSs are delivered via inhaled air to the target organ and they reach the systemic circulation either by direct absorption through the respiratory epithelium or via the gastrointestinal tract after unintentional ingestion (Derendorf et al., 1998; Donnelly and Seale, 2001). The swallowed proportion of the drug is, however, almost completely inactivated by the effective first-pass hepatic metabolism (99% for FP and 90% for budesonide (BUD)) (Ryrfeldt et al., 1979; Harding, 1990; Lipworth and Jackson, 1999).

The systemic adverse effects of inhaled FP and oral prednisone have already been studied in healthy dogs, with less HPA axis suppression when using FP compared to prednisone (Cohn et al., 2008). There do not appear to be any reports describing the systemic adverse effects of inhaled BUD, whereas significant suppression of the HPA axis has been detected with oral BUD at a dose of 3 mg/m^2 for 30 days in dogs with inflammatory bowel disease (Tumulty et al., 2004). In humans, the long-term use of FP has been found to cause dose-related adrenal suppression more often than BUD at equal dosages (Clark et al., 1996; Clark and Lipworth, 1997; Lipworth, 1999; Kaliner, 2006). The objective of the current study was to compare the endocrine effects of inhaled BUD, inhaled FP and oral prednisolone in healthy Beagle dogs at clinically relevant doses.

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http://dx.doi.org/10.1016/j.tvjl.2012.04.029

Materials and methods

Dogs and verification of health

Six healthy neutered female laboratory Beagle dogs were enrolled in the study. At the start of the study, the dogs were 2.5 years old and their mean bodyweight (BW) was 17 kg (range: 11–19 kg). The health status of each dog was assessed by thorough physical examination, haematology and serum biochemistry panels (alanine aminotransferase, albumin, alkaline phosphatase (ALP), blood urea nitrogen, calcium, creatinine, glucose, potassium, protein, sodium and total bilirubin) as well as by urinalysis (bacterial culture, protein-to-creatinine ratio, reagent strip (Multistix 10 SG, Siemens Healthcare Diagnostics), sediment and specific gravity). Laterolateral and ventro-dorsal thoracic radiographs were taken. Additionally, faecal flotation and sedimentation tests were performed.

The study protocol was approved by the Committee of Experimental Animals of Western Finland.

Study design

This study was an open label, prospective, randomised, placebo-controlled, cross-over design. All dogs received all of the following four treatment protocols in random order: (1) BUD inhalation (Pulmicort HFA, AstraZeneca) 200 μg twice daily; (2) FP inhalation (Flixotide Evohaler, GlaxoSmithKline) 250 μg twice daily; (3) prednisolone orally (Prednisolon, Leiras) 1 mg/kg once daily; (4) placebo inhalation twice daily. Inhaled medications were administered through an aerosol chamber (AeroDawg, Trudell Medical International) attached to an anaesthetic mask (face mask with rubber diaphragm, Kruuse). Before each medication, the metered-dose inhaler (MDI) was shaken and attached tightly to the aerosol chamber. An anaesthetic mask was then fitted to cover the dog's nose and mouth, the medication was sprayed into the chamber and eight breaths were counted. The placebo group breathed room air through the chamber and mask.

All dogs had their own masks, aerosol chambers and MDIs during the 4-week study period, and the inhalation devices were carefully washed after each treatment period. The last medication was given 24 h before blood sampling. Blood samples (serum and K_2EDTA samples, 6 mL each) were taken at the same time of the day (between 09:00 and 11:00 h) in the same environment where the dogs were housed. To avoid the possible effect of circadian rhythm on cortisol excretion (Derendorf, 1997), as well as stress-induced elevation in cortisol levels, we standardised the environmental factors and sample-taking protocol as far as possible, by constant sampling time and place and the same two persons collecting the samples.

Each treatment and each washout period lasted 4 weeks. The health status was assessed on days 0, 14, 28 and 35 by physical examination, haematology, serum biochemistry and urinalysis, and additionally by ACTH stimulation test on days 0, 28 and 35. All blood samples were taken from the cephalic vein and urine samples by cystocentesis using ultrasonographic guidance. All haematological, serum biochemical and urinalysis samples were stored at room temperature and analysed within 2 h.

ACTH stimulation test

The adrenocorticotropic hormone (ACTH) stimulation test is the most reliable way to diagnose adrenal dysfunction in dogs (Herrtage, 2005; Lathan et al., 2008), and in our study the adrenocorticotropic status was assessed with the low-dose ACTH stimulation test (Kerl et al., 1999; Frank et al., 2004) using a synthetic analogue of ACTH (Synacthen, Alliance; 5 μg/kg IV) administered via a plastic catheter (Optiva 20 G, Smiths Medical). The catheter was immediately flushed with 5 mL of physiological saline and a second blood sample was taken 60 min later. Serum samples for cortisol assays were frozen at −80 °C until analysis.

Cortisol assays were performed by a separate technician who was blinded to the treatments. Samples were analysed in duplicate with a commercial radioimmunoassay (RIA) kit validated for canine use (Coat-A-Count Cortisol, Siemens Healthcare Diagnostics). The intra-assay coefficient of variation (CV) for serum cortisol determination was 5.6% at the mean concentration level of 42.6 nmol/L and 6.4% at the level of 280.2 nmol/L (both CVs were calculated from 10 duplicate determinations). The samples were analysed in one series, and no inter-assay CV% was determined. The detection limit of the serum cortisol assay was 6.9 nmol/L.

Statistical analysis

The effect of treatments on the continuous response variables was assessed using a linear mixed effects model, where treatment, period, day of period, and interaction terms between treatment and period, and treatment and day of period were used as fixed effects and dog as a random effect. With urine protein-to-creatinine ratio and urine-specific gravity, the dog-related variance component was very close to zero. To get the model to converge, linear analysis was performed, excluding the very small random effect of individual dogs.

Carry-over effect was excluded from all models, as the day 0 ACTH-stimulated cortisol results proved that the 4-week wash-out was sufficiently long to normalise the HPA axis function before the next period. Data were expressed as means ± SD and range. A P-value <0.05 was considered significant. All statistical analyses were performed at 4Pharma using SAS System for Windows, version 9.2 (SAS Institute).

Results

Dogs and verification of health

All of the dogs showed normal physical examination results. No significant changes were detected in thoracic radiographs, and all faecal tests to detect parasites were negative. Results on days 0, 14, 28 and 35 of haematological analyses, including differential cell counts, were within the reference range in all dogs with minor exceptions. Serum biochemistry panels and urinalysis results were within the reference range, except changes detected in serum ALP and protein values and urine protein-to-creatinine ratio in the prednisolone group on days 14 and 28 (Table 1).

ACTH stimulation test

Test results are presented in Table 2. On day 0, no significant differences were seen in baseline or ACTH-stimulated peak cortisol levels when treatment groups were compared with the placebo group. Furthermore, no significant changes were noted in the placebo or BUD groups in basal or peak cortisol levels after 4 weeks of treatment relative to day 0 ($P > 0.05$ in each case). However, on day 28, prednisolone had significantly suppressed the basal cortisol level compared with the placebo ($P = 0.016$) or BUD ($P = 0.029$) group (Fig. 1). Additionally, the peak cortisol level after the ACTH stimulation test was significantly lower in the prednisolone and FP groups than in the placebo or BUD group (Fig. 2; $P < 0.001$ in each case).

One week after discontinuation of treatment (day 35) in the FP group, significant elevation of the basal cortisol level was found compared with day 28 ($P < 0.001$). The level was also higher than that in the placebo group (Fig. 1; $P = 0.035$), but no significant change was observed compared with the basal cortisol level in the FP group on day 0 ($P = 0.099$). On day 35, no significant differences between the treatment groups were detected in ACTH-stimulated peak cortisol concentrations (Fig. 2).

Table 1
Serum biochemistry and urinalysis results (mean ± SD) in the four treatment groups ($n = 6$ per group).

	Day 0	Day 14	Day 28	Day 35
Placebo				
ALP (U/L)	110 ± 43	107 ± 48	114 ± 46	109 ± 46
Prot (g/L)	57 ± 3	55 ± 4.7	57 ± 5.7	55 ± 1.8
U pr/cr	0.08 ± 0.02	0.11 ± 0.02	0.11 ± 0.02	0.10 ± 0.02
Prednisolone				
ALP (U/L)	105 ± 45	261 ± 91*	246 ± 101*	119 ± 43
Prot (g/L)	58 ± 2.1	65 ± 3.4*	65 ± 2.3*	57 ± 3.0
U pr/cr	0.08 ± 0.02	0.15 ± 0.04	0.47 ± 0.33*	0.15 ± 0.03
FP				
ALP (U/L)	115 ± 47	93 ± 19	99 ± 22	103 ± 34
Prot (g/L)	58 ± 2.3	58 ± 2.3	58 ± 2.7	55 ± 3.1
U pr/cr	0.10 ± 0.04	0.10 ± 0.03	0.16 ± 0.12	0.10 ± 0.02
BUD				
ALP (U/L)	100 ± 25	107 ± 28	117 ± 38	107 ± 42
Prot (g/L)	56 ± 3.5	57 ± 2.8	58 ± 1.6	55 ± 2.9
U pr/cr	0.09 ± 0.03	0.12 ± 0.03	0.11 ± 0.02	0.10 ± 0.02

FP, fluticasone propionate; BUD, budesonide; ALP alkaline phosphatase (reference range, 33–215 U/L); prot, protein (reference range, 58–77 g/L); U pr/cr, urine protein-to-creatinine ratio (reference range, <0.5).
* Significant difference compared to placebo, $P < 0.001$.