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Short-term prednisolone therapy has minimal impact on calcium metabolism in dogs with atopic dermatitis

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ABSTRACT

Glucocorticoids (GCs) are a large group of drugs used to treat a range of inflammatory, autoimmune and neoplastic diseases in dogs. Glucocorticoids have been linked to disturbances in calcium metabolism and skeletal disorders in humans, yet their effects at therapeutically effective dosages in dogs with spontaneous diseases are poorly understood. Serum concentrations of calcium, phosphate, vitamin D metabolites and plasma concentrations of parathyroid hormone and ionised calcium together with urinary fractional excretion of calcium and phosphate, were measured in 16 dogs with atopic dermatitis before and 6 weeks after standard dosage prednisolone treatment (0.93–1.06 mg/kg) every other day after 7 days of treatment with the same dosage once daily.

The severity of their physical signs, as assessed by the canine atopic dermatitis extent and severity index version 3 (CADESI-03) and the Edinburgh Pruritus Scale (EPS), decreased in all dogs following prednisolone treatment. There was no significant difference in any of the biochemical parameters measured following prednisolone treatment. This study indicates that prednisolone, used at a therapeutically effective dose, has minimal impact on calcium metabolism in dogs with atopic dermatitis.

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Introduction

Canine atopic dermatitis (AD) is defined as an inflammatory and pruritic allergic skin disease with characteristic clinical features associated with IgE antibodies most commonly directed to environmental allergens (Halliwell, 2006). Currently, medications with good evidence of efficacy include topical and systemic glucocorticoids (GCs) and calcineurin inhibitors such as oral ciclosporin and topical tacrolimus (Olivry et al., 2010a). Allergen specific immunotherapy may also be helpful in reducing recurrence of physical signs upon subsequent exposure to environmental allergens to which the patient is hypersensitive (Olivry et al., 2010b). Outcomes of randomized controlled trials (RCTs) by recent systematic reviews indicated that the oral GCs (prednisone, prednisolone or methylprednisolone) are effective for the treatment of canine AD (Olivry et al., 2010b). However, GCs can be associated with a range of adverse effects including polyuria, polydipsia, polyphagia and opportunistic microbial infections (Olivry and Sousa, 2001; Cohn, 2010).

In contrast to the apparent situation in dogs, osteoporosis and associated pathological fractures are some of the most serious and debilitating adverse effects associated with GC treatment in humans (Weinstein, 2001; De Vries et al., 2007). As they are such an important cause of morbidity in human medicine, it is widely recommended that many patients on GCs receive therapies directed at minimising the risk of osteoporosis and fracture (Gourlay et al., 2007). However, despite the importance of bone loss in humans treated with GCs, the precise mechanism(s) of GC-associated osteoporosis is unknown (McLaughlin et al., 2002; Rubin and Bilezikian, 2002; Mazziotti et al., 2006; Weinstein, 2010).

Parathyroid hormone (PTH) and vitamin D play a crucial role in calcium metabolism and maintenance of skeletal structure. The active hormonal form of vitamin D, 1,25 dihydroxyvitamin D (active vitamin D₃, calcitriol, 1,25 dihydroxycholecalciferol, 1,25 dihydroxyvitamin D₃, 1,25(OH)₂D₃ or simply 1,25(OH)₂D), is necessary for optimal intestinal absorption of calcium and phosphorus and also exerts an inhibitory effect on PTH synthesis (Lips, 2001). Vitamin D deficiency and secondary hyperparathyroidism can contribute to accelerated bone loss and increased fragility (Bischoff-Ferrari et al., 2004; Sambrook et al., 2004). Clinical trials involving older people at high risk for calcium and vitamin D deficiency indicate that supplementation of both can reverse secondary hyperparathyroidism, decrease bone resorption, increase bone mass,

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and decrease fracture rates (Lips, 2001). The decrease in vitamin D and increase in PTH concentrations during the winter months is associated with an increase in number of fractures, independent of the increase in rate of falls (Pasco et al., 2004).

Since PTH and 1,25(OH)₂D play a pivotal role in the maintenance of skeletal tissue and GCs are associated with skeletal pathology in humans, a number of studies have examined the effects of GCs on their circulating concentrations. For example, studies examining their effects on plasma PTH concentrations include those of Hattersley et al. (1994), Paz-Pacheco et al. (1995) and Rubin and Bilezikian (2002) and on serum vitamin D concentrations those of Hahn et al. (1979) and Prummel et al. (1991). In contrast, there have been few studies on the effect of GCs, used at therapeutically effective dosages, on calcium metabolism in dogs with spontaneous diseases (Garrett et al., 1964; Quarles, 1992). This is particularly surprising given that reports have recently shown that dogs with spontaneous disorders of glucocorticoid metabolism often have altered calcium metabolism (Peterson et al., 1996; Ramsey et al., 2005; Gow et al., 2009). Consequently, we hypothesised that a therapeutically effective, every-other-day treatment regime of prednisolone would lead to significant increases in plasma PTH concentrations in dogs with AD.

The primary aim of this study was to assess the effect of prednisolone used at standard therapeutically recommended dosages (Scott et al., 2001), on calcium metabolism in dogs with AD. We measured serum calcium phosphate, albumin, the prehormone 25 hydroxyvitamin D (calcitriol, or simply 25(OH)D), 1,25(OH)₂D and PTH and ionised calcium concentrations together with urinary fractional excretion of calcium and phosphate before and 6 weeks after prednisolone treatment at approximately 1 mg/kg every other day after a week's induction dosage of approximately 1 mg/kg/day. In order to confirm that this treatment regimen was effective clinically, we also evaluated the severity of the physical signs before and after treatment.

Materials and methods

Animals

Sixteen dogs were enrolled in the study comprising cross breeds (2), Jack Russell terrier (2), German shepherd dog (2), Bullmastiff (1), West Highland white terrier (2), Cavalier King Charles spaniel (1), English setter (1), Japanese Akita (1), boxer (1), Airedale terrier (1), Labrador retriever (1) and Staffordshire bull terrier (1). Nine were male (four castrated) and seven were female (six ovariectomised). Their ages ranged between 1.5 and 8.5 years (median 3.3 years) and they weighed between 6.8 kg and 52.0 kg (median 23.8 kg).

Study design

This study was undertaken in the Dermatology Unit of the Royal (Dick) School of Veterinary Studies (R(D)SVS), University of Edinburgh, between January 2009 and January 2010. Consecutive dogs with a clinical diagnosis of AD according to well-established criteria (DeBoer and Hillier, 2001) were considered eligible for the study. A standardised diagnostic procedure for the diagnosis of atopic was used, including laboratory tests for the diagnosis of ectoparasitic infections, cytological examinations for bacterial and yeast infections, fungal culture to rule out dermatophytosis, skin biopsy and histological examination of tissues to rule out diseases such as cutaneous lymphoma or sebaceous adenitis. Cutaneous adverse food reactions (CAFRs) were ruled out by feeding suitable home or commercially prepared selected diets as indicated by the dietary history of the dog for at least 8 weeks. Dogs with physical and laboratory evidence of secondary microbial infections, e.g. bacterial pyoderma or *Malassezia* dermatitis, were treated to resolution prior to inclusion and, using only topical therapy as required, if relapses occurred during the study. In addition, dogs with AD that had received GCs by any route in the previous 12 weeks were excluded.

Following a standard therapeutic cascade, consecutive atopic dogs were treated with prednisolone if the preparation of an allergen-specific immunotherapy vaccine was pending or immunotherapy had been discontinued due to lack of efficacy. No other treatments were allowed. A total of 16 consecutive atopic patients were selected to receive oral prednisolone (Prednicare, Animalcare Limited) at as near as

possible to the recommended dosage of 1 mg/kg (actual dosage, 0.93–1.06 mg/kg). All dogs initially received this once daily for 7 days and subsequently every other day for the following 5 weeks.

Serum concentrations of calcium, phosphate, creatinine, 25(OH)D and 1,25(OH)₂D together with plasma PTH and ionised calcium concentrations were measured after a 12 h fast prior to prednisolone administration and then again after a total of 7 weeks of treatment. In addition, 12 h fasting free catch urine samples were collected before and after treatment and urinary creatinine, calcium and phosphate concentrations were determined. The venous blood samples for measurement of serum calcium, phosphate, creatinine, albumin, 25(OH)D and 1,25(OH)₂D were collected into plain tubes and allowed to clot at room temperature before centrifugation and harvesting of serum. Samples for plasma ionised calcium were collected into lithium heparin tubes. Samples for PTH determinations were harvested into ice-cold EDTA tubes. Plasma samples were centrifuged immediately and were stored at –20 °C within 7 min and at –80 °C within 1 h of sampling until shipment in cold packs for subsequent analyses.

The plasma PTH concentrations were measured using a two-site immunoradiometric assay (Diagnostic Systems Laboratory, Beckman). The intra- and inter-assay coefficients of variation were <3.5%, and <5.2%, respectively. Samples for analysis of urinary calcium, phosphate and creatinine were collected for assessment at the same time and stored at –80 °C until analysis. Phosphate was analysed by measuring the formation of unreduced phosphomolybdate complex, which is proportional to the amount of inorganic phosphorus in the sample. Calcium was analysed using bichromatic analysis of calcium in the sample and o-cresolphthalein complexone in alkaline solution. Serum phosphate, calcium and creatinine were analysed on the day of blood sampling using an IL600 analyser (Instrumentation Laboratories). Ionised calcium was measured using an AVL 9180 electrolyte analyser. Ionised calcium concentrations were collected anaerobically and analysed within 20 min of collection.

Analyses of serum 25(OH)D and 1,25(OH)₂D concentrations were carried out in batch modes as described in detail elsewhere (Mawer et al., 1985, 1990). Briefly, samples were extracted using acetonitrile and applied to C18 Silica Sep-paks. Separation of metabolites was by straight phase high-performance liquid chromatography (HPLC) (Waters Associates, Milford) using a Hewlett-Packard Zorbax-Sil Column (Hichrom, Reading) eluted with hexane:propan-2-ol:methanol (92:4:4). Serum 25(OH)D2 and 25(OH)D3 concentrations were measured separately by application to a second Zorbax-Sil Column eluted with hexane:propan-2-ol (98:2) and quantified by ultra violet (UV) absorbance at 265 nm and corrected for recovery (sensitivity 2 ng/mL [5 nmol/L], intra- and inter-assay coefficients of variation were 3.0% and 4.2%, respectively) (Berry et al., 2000). Results are expressed as total 25(OH)D.

Following separation by high performance liquid chromatography (HPLC), 1,25(OH)₂D concentrations were quantified by radioimmunoassay as described in detail elsewhere (sensitivity 1.25 pg [3 pmol], intra- and inter-assay coefficients of variation 7.8% and 10.5%, respectively) (Mawer et al., 1990). The assay laboratory is accredited to the International Organisation of Standardization (ISO) 9001:2000 and ISO 13485:2003 and participates successfully in the Vitamin D external quality assessment scheme (DEQAS).

In order to assess that the response to treatment was clinically acceptable, the skin lesions of all dogs were assessed at day 0 and a median of 42 days later (range 40–45 days) using the canine AD extent and severity index (CADESI)-03 as previously described (Olivry et al., 2007). The owners used the Edinburgh Pruritus Scale to score the pruritus on a numerical scale (0–5) annotated with specific behavioural descriptors as previously described by our group (Colombo et al., 2005).

Investigations were performed in client-owned dogs with owner permission. The study was approved by the University of Edinburgh's Ethical Review Committee through the R(D)SVS's Veterinary Ethical Review Committee.

Statistical analyses

The effect of treatment on the concentrations of calcium, ionised calcium, phosphate, 25(OH)D, 1,25(OH)₂D, PTH and urinary fractional excretion of phosphate and calcium were tested with Wilcoxon matched pairs tests. A *P*-value of ≤0.05 was used as the criterion for statistical significance. Statistical analyses were performed by GraphPad Prism (GraphPad Software, La Jolla).

Results

All dogs completed the study. Adverse effects of prednisolone treatment were seen in all 16 dogs. These consisted of polyuria/polydipsia (16/16, 100%) and polyphagia (15/16, 93.75%); all the signs were mild and reduced/resolved rapidly after the medication was titrated downwards to the lowest acceptable dosage/discontinued subsequently once the 6 week study period had elapsed. The 16 dogs were a subset of the dogs previously reported in Kovalik et al. (2011).

Table 1

Serum, plasma and urine pre-treatment and post-treatment median parameters and statistical comparisons.

Parameter	Reference range	Pre-treatment median (range)	Post-treatment median (range)	P-value
Serum calcium (mmol/L)	2.3–3.0	2.49 (2.28–2.73)	2.56 (2.35–2.97)	0.28
Plasma ionised calcium (mmol/L)	1.15–1.50	1.32 (1.12–1.46)	1.34 (1.25–1.43)	0.46
Serum phosphate (mmol/L)	0.9–2.0	1.33 (1.11–2.64)	1.26 (1.01–3.93)	0.06
Serum creatinine (μ mol/L)	40.0–132.0	99.0 (79–131)	94.0 (72–119)	0.30
Urinary FE calcium (%)	0.03–0.22	0.09 (0.02–0.36)	0.13 (0.02–0.52)	0.16
Urinary FE phosphate (%)	0.4–20.1	8.1 (0.14–27.91)	10.7 (1.65–27.24)	0.09
Plasma PTH (pg/mL)	20–65	31.5 (10.0–130.0)	38.0 (15.0–229.0)	0.73
Serum 25(OH)D (ng/mL) ^a	19.3–43.6	30.5 (18.2–44.1)	27.4 (19.0–41.5)	0.21
Serum 1,25(OH) ₂ D (pg/mL) ^a	16.0–38.0	41.5 (18.0–66.0)	39.0 (20.0–77.0)	0.90

FE, fractional excretion; PTH, parathyroid hormone; 25(OH)D, 25 hydroxyvitamin D; 1,25(OH)₂D, 1,25-dihydroxyvitamin D₃.^a Preliminary reference range provided (Gow et al., 2009).

The median serum or plasma concentrations of the biochemical parameters measured are listed in Table 1. There were no significant differences in serum concentrations of calcium, phosphate, creatinine, 25(OH)D and 1,25(OH)₂D before and after treatment. In addition, there were no significant differences in plasma ionised calcium or PTH concentrations or urinary fractional excretion of calcium and phosphate following treatment.

Prednisolone treatment reduced CADESI-03 scores by $\geq 50\%$ in all 16 dogs (100%). Comparison of EPS scores on days 0 and 42 indicated that treatment with prednisolone reduced pruritus by $\geq 50\%$ in all 16 dogs (100%), both criteria indicating a satisfactory physical response.

Discussion

The central finding of this study was that prednisolone administered at a therapeutically effective dosage had minimal effects on calcium metabolism in dogs with AD. This is in agreement with a previous study by Quarles (1992) who found no significant changes in PTH or ionised calcium concentrations in 15 Beagles that received 1.3 mg/kg of prednisolone administered as a single oral dosage once daily for 29 weeks. However, the findings in the present study are in contrast to those of Ramsey et al. (2005) who found that PTH was significantly raised in a cohort of dogs with naturally-occurring hyperadrenocorticism (HAC). The reason(s) why this dosing regime of prednisolone, in contrast to dogs with HAC, was/were not associated with a significant increase in PTH concentrations is unclear but may be related to the dosage administered, intermittent (every other day) dosing strategy, duration of the treatment or to the timing of PTH measurement which was 24 h after prednisolone dosing.

The findings regarding plasma PTH concentrations in this study are similar to those reported in humans. Whilst some studies have found that PTH concentrations increase following GC therapy (Fucik et al., 1975; Gennari et al., 1984), there is a growing consensus that PTH concentrations are not significantly increased following GC treatment (Seeman et al., 1980; Hahn et al., 1981; Prummel et al., 1991). Bone loss in human patients with primary hyperparathyroidism tends to be in cortical sites whereas that in patients treated with GCs occurs more commonly in cancellous bone (Silverberg et al., 1989). This also supports the hypothesis that increased PTH concentrations are unlikely to be critical for GC associated osteoporosis (Reid et al., 1992).

Our observation that prednisolone administered at a therapeutically effective dosage did not alter calcium concentrations is consistent with earlier studies in humans (Fucik et al., 1975; Ekenstam et al., 1988). Evaluation of the chronic and acute administration of excessive doses of GCs to 11 healthy adult men revealed no significant changes in serum calcium concentrations (Fucik et al., 1975). Additionally, nine asthmatics treated with high intravenous dosages of betamethasone (0.65 mg/h) had no significant changes in

serum calcium and phosphate concentrations (Ekenstam et al., 1988).

We found no significant alterations in vitamin D metabolism following the administration of prednisolone. Similar findings have been reported in humans; for example, serum 25(OH)D concentrations in people receiving chronic supraphysiological dosages of prednisone (mean 22 mg/day) were not significantly different when compared with age-matched controls (Hahn et al., 1979). In another study, Hahn et al. (1979) found no change in serum 25(OH)D concentrations after the administration of daily dosages of 20 mg of prednisolone for 2 weeks. Prummel et al. (1991) reported similar findings in 10 euthyroid patients with Graves' ophthalmopathy, after a 12-week course of prednisone.

We observed no differences in urinary calcium and phosphate excretion following prednisolone treatment, which is in agreement with a study by Yonemura et al. (1999) who found no significant urinary excretion of calcium in 15 people treated with 40 mg prednisolone daily. Additionally, 10 mg prednisolone administered once daily administered to healthy cats for 2 weeks was also not associated with increased urinary calcium excretion (Geyer et al., 2007).

The limitations of this study include the relatively small number of dogs. However, we have also examined the effects of ciclosporin on calcium metabolism, using the same number of dogs, which found that ciclosporin therapy was associated with a significant increase in plasma PTH concentrations (Kovalik et al., 2011). Consequently, the approach undertaken in this study have the power to detect differences in calcium metabolism, even in a relatively small number of dogs and the findings of no significant changes suggests that even if larger cohorts were recruited, any changes in biochemical parameters, if present, would be modest. However, we evaluated only one time point post-treatment and additional samples taken at different times following prednisolone therapy would undoubtedly offer further insights into its effects on calcium metabolism in dogs.

Conclusions

This study revealed that prednisolone, used at a therapeutically effective dosage and administered for 6 weeks, has minimal impact on calcium metabolism in dogs with AD. Additional, larger studies examining a similar range of biochemical parameters over a wider range of time points are required to further characterise the effect of prednisolone on calcium metabolism in dogs.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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