PAPER

Vitamin D shows in vivo efficacy in a placebo-controlled, double-blinded, randomised clinical trial on canine atopic dermatitis

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Abstract

Atopic dermatitis (AD) in dogs is among the most common skin diseases in small animal practice. It is an inflammatory disease based on a genetic predisposition to develop hypersensitivity against environmental and food allergens and typical clinical signs up exposure. Treatment sometimes can be difficult and associated with adverse effects. Previous studies evaluating cholecalciferol as treatment for human AD have shown promising results. With canine AD being a good animal model for its human counterpart, it was hypothesised that cholecalciferol might have beneficial clinical effects in dogs, too. In this randomised, placebo-controlled, double-blinded eight-week cross-over study, 23 client-owned dogs received either systemic cholecalciferol (n=16), a vitamin D receptor analogue (n=8) or placebo (n=13). Blood samples for ionised calcium were obtained regularly during the study, and Canine Atopic Dermatitis Extent and Severity Index and pruritus scores, blood levels of vitamin D metabolites, measurements of skin pH and transepidermal water loss were determined before and after. Pruritus and lesion scores decreased significantly in the cholecalciferol group versus placebo. No differences in water loss or skin pH were observed. An increase in serum 25-hydroxycholecalciferol strongly correlated with a reduction in pruritus. Systemic cholecalciferol may be a viable treatment option for canine AD.

Introduction

Atopic dermatitis (AD) in dogs is a frequent inflammatory and pruritic skin disease with characteristic clinical signs.[1 2] A genetic predisposition to develop a hypersensitivity against environmental and food antigens is assumed.[3] Environmental factors also play an important role in the development of AD in human beings and animals.[4] These genetic and environmental factors lead to changes in the epidermal barrier function and the immune response.[3 5] Canine AD is considered an excellent animal model for its human counterpart.[6–9]

The most prominent clinical sign of canine AD is pruritus; erythema or papules may be observed as the sole sign in early stages of the disease. Alopecia, crusts, hyperpigmentation and lichenification develop secondary to self-trauma and/or infection.[10] Spontaneous remission is rare,[11] and the disease prominently affects the owner's quality of life,[12] comparable to parents of atopic children.[13] Treatment typically consists of allergen avoidance, allergen-specific immunotherapy and symptomatic therapy with anti-inflammatory and antipruritic agents either individually or in combination.[14] The clinical course and treatment can be frustrating.[15]

Three major factors influence the pathogenesis of AD in human beings and dogs: a compromised skin barrier facilitates penetration of allergens into the skin,[16] an exaggerated immune response to those allergens is responsible for cutaneous inflammation[3] and the inability to control microorganisms on the surface leads to secondary microbial overgrowth.[17 18] Vitamin D is involved in the barrier function, the immune response and the production of antimicrobial peptides (AMP),[19 20] thus being instrumental in influencing bacterial growth on the surface of the skin.

Vitamin D is a highly potent hormone, and severe adverse effects such as hypercalcaemia have been associated with cholecalciferol therapy in human beings.[21] To minimise these adverse effects, an entire spectrum

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of vitamin D receptor (VDR) analogues has been developed which show a much better safety profile in human beings than cholecalciferol, while maintaining clinical efficacy.[22] Paricalcitol is a VDR agonist with potent immunomodulatory efficacy used in human beings[23] with a decreased potential to induce hypercalcaemia and hyperphosphatemia.[24]

25-Hydroxycholecalciferol (25-OH-D3) is the first metabolite of cholecalciferol following hydroxylation in the liver and is one of the most reliable vitamin D metabolites in laboratory evaluation.[25] Its serum concentration has been directly correlated to the severity of AD and atopic asthma in children.[26 27] Limited data are available in the atopic dog regarding the metabolisation of cholecalciferol when administered at high doses, although one report concerning oncology patients identified only low adverse effects.[28] The kidneys hydroxylate 25-OH-D3 to either the active 1,25-dihydroxycholecalciferol or to the 24,25-dihydroxycholecalciferol (24,25-OH-D3) metabolite. The latter is less likely to result in hypercalcaemia due to its weaker stimulation of osteoclasts. With high serum concentrations of 1,25-dihydroxycholecalciferol, the 25-OH-D3 is preferentially metabolised to the 24,25-OH-D3.[29] The enzyme 1,25-24-hydroxylase is responsible for this metabolisation and a deficiency thereof induces nephrolithiasis in human patients.[30]

The aim of this randomised, double-blinded, cross-over, placebo-controlled study was to evaluate whether vitamin D and the VDR analogue paricalcitol improve clinical signs and skin barrier function of canine AD. A further aim was to correlate serum concentrations of vitamin D and its metabolites to the clinical signs.

Methods

Study design

This study was originally planned as a three-arm randomised, double-blinded, controlled, cross-over trial comparing treatment of canine AD with cholecalciferol, paricalcitol or placebo with a washout period of eight weeks between treatments. Pre results, the trial was approved by governmental authorities (Government of Upper Bavaria) under the number 55.2-1-54-2532-99-12. After the first 13 dogs had been included, the protocol was changed to a two-arm randomised, double-blinded, cross-over trial due to adverse reactions to paricalcitol.

Study objects

Twenty-three pruritic dogs with confirmed AD were included. The diagnosis was based on clinical history, examination and ruling out differential diagnoses. Owners had to sign a written consent form informing them about possible side effects and crucial criteria for the study. Additional inclusion criteria included flea control for at least eight weeks before the start of the study and a novel single-protein elimination diet for at least six weeks without any clinical improvement. All dogs were on the same commercially formulated diets before and for the duration of the trial. Ongoing therapy for AD was only permitted if it was unchanged and had been administered for at least four weeks before inclusion with antihistamines, fatty acid supplementation or topical therapy and for at least eight weeks before in the case of immunosuppressive drugs such as cyclosporine or glucocorticoids. Allergen immunotherapy had to be ongoing for at least 12 months, and during the last three months before inclusion the dose and frequency of injections had to be constant. A dose change was not permitted during the study for all medications. One dog was on long-term glucocorticoid therapy alone, another dog was on a combination of dexamethasone and allergen immunotherapy (more than two years), one dog was on a combination of cyclosporine and allergen immunotherapy (more than one year) and three dogs were on allergen immunotherapy alone for over two years. Three additional dogs were on long-term antihistamine therapy, all other 14 patients received no adjunctive medication, apart from topical therapy such as shampooing or essential fatty acids (unchanged for more than six months). Supplementary sources of calcium such as bones or calcium powder were not permitted during the study to reduce the risk of hypercalcaemia.

Study intervention

Once daily, dogs received either placebo (palm oil), cholecalciferol (Vigantol-Oel, Merck KGaA, Darmstadt, Germany) at 300 IU/kg or the VDR agonist paricalcitol (Zemplar, Abbott Laboratories, Chicago, USA) at 0.02 µg/kg orally for eight weeks. The dosage was increased every two weeks based on the patient's blood calcium concentrations and the severity of clinical signs, from approximately 300 IU/kg to 700 IU/kg to 1400 IU/kg cholecalciferol and from 0.02 µg/kg to 0.04 µg/kg to 0.1 µg/kg paricalcitol by week 4. In one dog, cholecalciferol was further increased to 2700 IU/kg in week 6 by the owner. If the ionised calcium concentration rose above 1.42 mmol/L the dosage was reduced to the previous well-tolerated dosage. If calcium levels were within the reference range (1.20–1.42 mmol/L) and clinical signs were not resolved, the study medication dose was increased. To ensure double-blinding in this study, the paricalcitol and cholecalciferol were both diluted in palm oil to achieve identical medication volumes to cholecalciferol by staff that was otherwise not involved in the study.

The primary study outcome was the decrease of Canine Atopic Dermatitis Extent and Severity Index-03 (CADESI-03) and Pruritus-VAS compared with placebo. Secondary study outcomes were changes in skin barrier function and patients' general condition (see Fig 1).

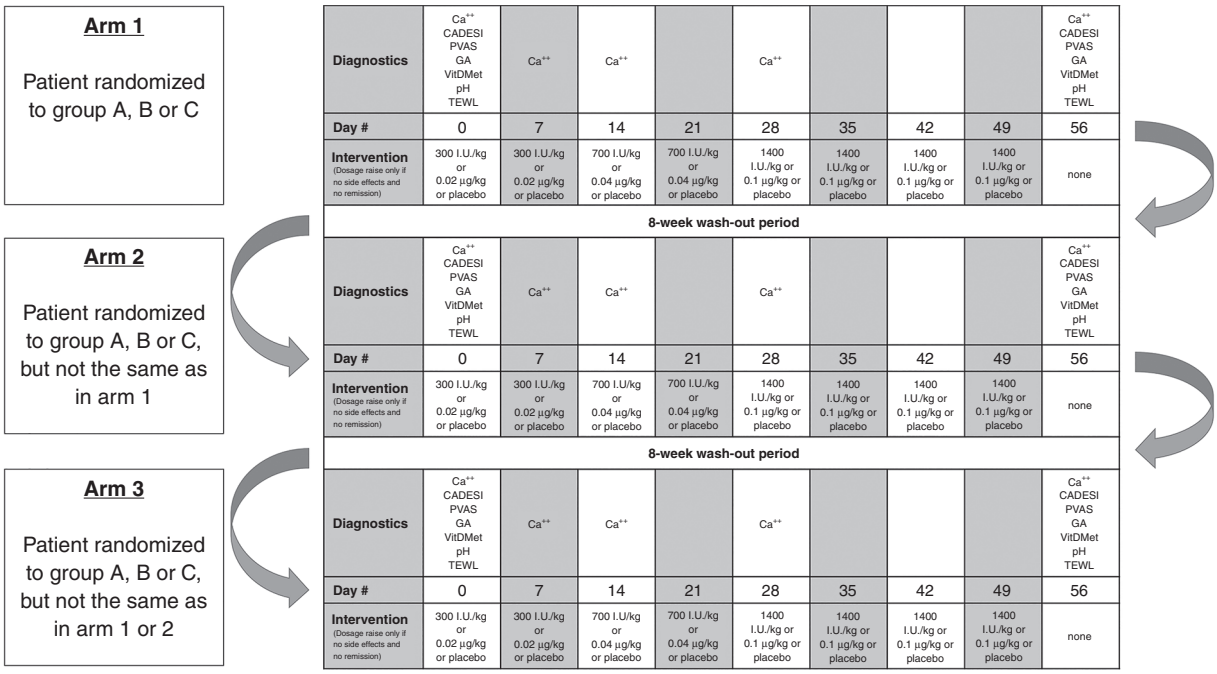

FIG 1: Study chart: tabular mapping of the study course with the three-arm protocol. CADESI, Canine Atopic Dermatitis Extent and Severity Index; GA, global assessment; PVAS, Pruritus Visual Analogue Scale; TEWL, transepidermal water loss.

Randomisation

The first 12 dogs were randomised to receive either placebo, cholecalciferol or paricalcitol. A computer-based randomisation matrix (Microsoft Excel 2011 for Mac, Microsoft, Redmont, USA) was used to determine the sequence of medications. Five dogs showed elevated ionised serum calcium, and after completion of that study arm treatment was unblinded for those dogs revealing paricalcitol as the cause of the adverse effects in four of the five dogs, the fifth dog developed hypercalcaemia on placebo. Consequently, the use of paricalcitol was discontinued and the remaining 11 dogs were only randomised to receive cholecalciferol or placebo.

Clinical evaluation

A validated lesion score (CADESI-03)[31] was obtained on days 0 and 56 by a blinded veterinary dermatology specialist. Additionally, on those days a pruritus score (Pruritus Visual Analogue Scale) designed and validated to measure the severity of itch in dogs was determined by the owners.[32] Owners were also asked for a global assessment (GA) of the treatment efficacy at the last visit. In this assessment, clients evaluated their dog's general condition as slightly (+1) or clearly improved (+2), slightly (−1) or clearly deteriorated (−2) or unchanged (0) compared with the first visit.

Further evaluations

The transepidermal water loss (TEWL) was determined with a closed-chamber vapometer (Delfin Technologies, Kuopio, Finland) and the skin pH with a pH meter (Mettler Toledo, Schwerzenbach, Switzerland), each by calculating the means of five individual measurements on each of four body sites (ventrum, tail base, inner pinna and between the shoulder blades) on days 0 and 56. The fur was clipped directly before measurement, and sampling was performed under adequate room temperature and humidity (according to the manufacturer's recommendations).

Blood samples were obtained and ionised calcium concentrations determined with blood gas analysis (Siemens RapidPoint 405, Siemens Healthcare, Munich, Germany) on days 0, 7, 14, 28 and 56 to monitor for hypercalcaemia. If adverse clinical changes such as polyuria, polydipsia or anorexia were observed, serum analysis and additional calcium measurements were performed and patients' medication was withdrawn for one day. If signs resolved, treatment was reinitiated at the dose administered before the occurrence of the adverse effects. If signs did not resolve completely within the week, patients were to be withdrawn from the study. At each visit, a complete dermatological examination was performed including cytology if needed.

Additionally, blood samples from day 0 and 56 were frozen at −80°C and sent frozen to a commercial laboratory (Labor Dr. Limbach & Kollegen, Heidelberg, Germany) for validated serum analysis of vitamin D metabolites, namely 25-OH-D3, 25-hydroxyergosterol (25-OH-D2), 24,25-OH-D3 and 3c-epimer-25-hydroxycholecalciferol (3c-E-25-OH-D3). Studies have shown liquid chromatography tandem mass spectrometry to be a reliable method of measuring vitamin D metabolites in human patients.[33 34]

Statistics

Based on a CADESI-03 SD of 18 (as reported in previous studies on canine AD), a power of 80 per cent and a significance level set at 0.05, it was calculated that 12 dogs per study arm were needed to detect a difference

of 15 points in the CADESI. After data collection, it was decided to only evaluate data of the arms using placebo and cholecalciferol due to the cessation of paricalcitol treatment during the study.

Data were evaluated for normality with a D'Agostino and Pearson omnibus normality test. Changes in CADESI-03, pruritus and serum concentrations of vitamin D and its metabolites between groups were compared with a Mann-Whitney test. Correlations between medication serum concentrations and clinical scores were calculated with a Spearman correlation test. To evaluate potential cross-over effects, the lesion scores of dogs before treatment with placebo first were compared with those of dogs treated with placebo after a course of vitamin D and the eight-week washout period. A P value <0.05 was considered significant. Analysis was performed with a commercial program (GraphPad Prism 6 for Mac, GraphPad Software, La Jolla, USA).

Results

Study objects and adverse effects

Twenty-three atopic dogs were included in the study. Only 2 dogs completed all three treatments, owners of 10 dogs discontinued the study after two treatment arms and 11 dogs received only one arm. In total, 37 treatment courses were conducted: 16 dogs received cholecalciferol, 13 dogs palm oil as placebo and 8 dogs were administered the VDR agonist paricalcitol. Five of those early courses resulted in polydipsia, polyuria and hypercalcaemia. The clinical signs resolved within one day after discontinuation of the medication, and all five patients restarted at the earlier safe dosage and completed the study arm with normal calcium levels and without further adverse clinical effects. Unblinding in those individuals occurred after each of those dogs had completed the study arm. Four of the five dogs had received the VDR agonist, the remaining patient was in the placebo group. As a result of these 4 dogs' reactions, the VDR agonist treatment arm was discontinued and the remaining 12 dogs were only treated with placebo and cholecalciferol. Most dogs responded well to 1000–1400 IU/kg. However, few dogs needed a higher dosage and in one dog 300 IU/kg was sufficient. There was no significant difference between the lesion scores of dogs before treatment with placebo first and those of dogs treated with placebo after a course of vitamin D, indicating no carry-over effects.

Clinical evaluation

After eight weeks of treatment, patients who received cholecalciferol showed significantly less pruritus than patients from the placebo group ($P<0.0001$, Fig 2). Also, 9 of 16 dogs (56.3 per cent) in the cholecalciferol group improved in their pruritus score by ≥50 per cent compared with 1 dog receiving placebo. The difference in decrease of CADESI scores from day 0 to 56 between placebo and cholecalciferol was highly significant ($P=0.0069$, Fig 3). GA scores did not change significantly. Also, 6 of 16 dogs (37.5 per cent) in the cholecalciferol group improved in their CADESI score by ≥50 per cent compared with 2 dogs in the placebo group. Mean, median and range of improvement in CADESI, pruritus and GA scores are listed in online supplementary table I.

TEWL and pH values

Neither TEWL nor pH changes were significant between any of the groups and time points. Mean, median and range of changes are listed in online supplementary table I.

Vitamin D metabolites

In total, 20 of the 23 participating dogs had 25-OH-D3 serum concentrations below 50 µg/L (17 of these even below 40 µg/L). Concentrations of serum vitamin D metabolites could have been measured in only 33 of the 37 clinical treatment courses. Two samples were lost during processing and in two cases there was not enough serum available for a validated analysis. Over the study period of eight weeks, 25-OH-D3 showed a mean increase of 80 µg/mL (250 per cent) in the cholecalciferol group (n=13; $P=0.0007$), while the

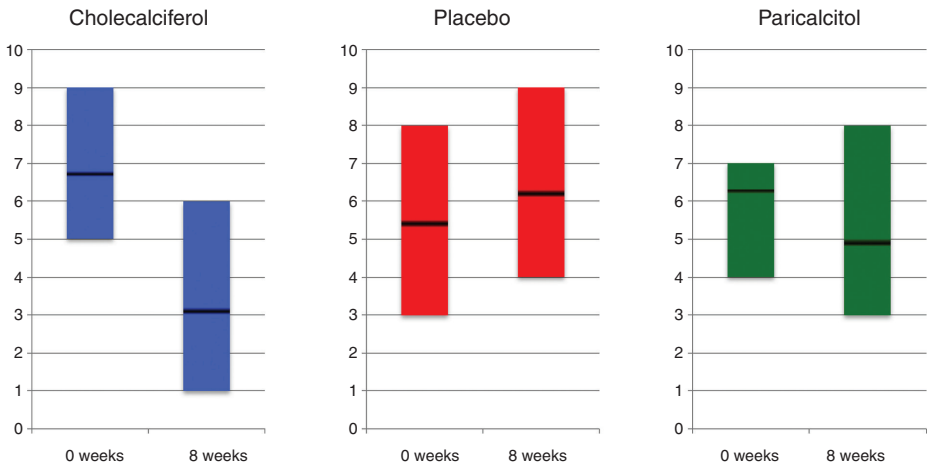

FIG 2: Pruritus Visual Analogue Scale in different groups (cholecalciferol, placebo, paricalcitol). Black lines mark median, and coloured boxes mark range of scores at zero and eight weeks of therapy.

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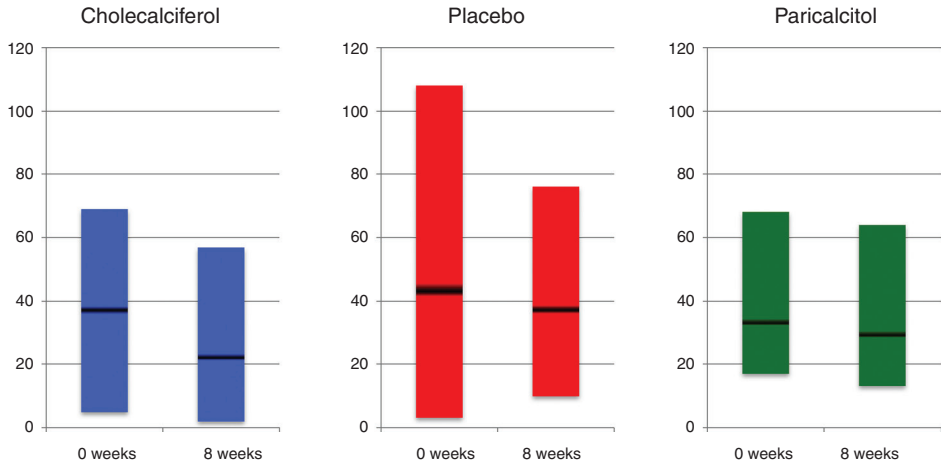

FIG 3: Canine Atopic Dermatitis Extent and Severity Index values in different groups (cholecalciferol, placebo, paricalcitol). Black lines mark median, and coloured boxes mark range of scores at zero and eight weeks of therapy.

paricalcitol group 25-OH-D3 concentrations increased by an average of 36 µg/mL (n=7; 105 per cent). The placebo group showed a decreased vitamin D by an average of 14 µg/mL (n=13; −20 per cent). 24,25-OH-D3 mean concentrations increased in the cholecalciferol group by 30 µg/mL (1200 per cent; P=0.0009) and in the paricalcitol group by 11.9 µg/mL (460 per cent), whereas it was again decreased in the placebo group by an average of 4.5 µg/mL (−10 per cent). No significant changes were observed in 25-OH-D2 or 3c-E-25-OH-D3. There was a statistical correlation between the decrease in pruritus-VAS and the increase in 25-OH-D3 (P=0.005, r=0.484) as well as the increase in 24,25-OH-D3 (P=0.008, r=0.46, supplementary table II).

Discussion

In this study, oral vitamin D decreased both pruritus and also acute and chronic skin lesions in dogs with AD. The improvement in the dogs' pruritus was significantly correlated with an increase in serum 25-OH-D3 as well as 24,25-OH-D3 levels. AD is one of the most common skin diseases in small animal practice[35] and is also of high prevalence in human beings.[36] In human patients, vitamin D has been reported as an important factor in the development and clinical course of AD, often associated with changes in AMP expression.[19 27 37] AMP are regarded as the first-line defence of the skin against secondary infections, especially in diseases with a reduced skin barrier function such as AD.[38 39] Especially the upregulation of cathelicidins promoted via the VDR is considered to play a major role in atopic lesions. In human AD, the concentrations of cathelicidins are significantly decreased compared with human psoriatic skin.[38 40 41] In dogs with AD, a first report identified similar changes in gene expression.[5] A specific VDR haplotype gene polymorphism change has been described in human AD; this gene is considered responsible for the regulation of the epidermal barrier function.[19] Furthermore, a study has shown an upregulation of transforming growth factor-β-dependent FoxP3- or interleukin-10-dependent regulatory T cells,[42] which might also influence AD symptoms in dogs.

In this first, double-blinded study evaluating vitamin D in dogs with canine AD, pruritus was significantly reduced after eight weeks of therapy compared with treatment with placebo.

Many human atopic individuals have demonstrated vitamin D serum concentrations below the reference range.[27 43] A recent study has shown an association between VDR gene polymorphism and severe AD in human adults, where a specific receptor haplotype is more common in atopic people.[19] Based on an unpublished pilot study by one of the collaborators (HK), it was hypothesised that AD in dogs may be associated with decreased vitamin D concentrations as has been described for human beings.[44] Before this study, the authors treated a case series of five atopic dogs with oral vitamin D (cholecalciferol) at 300–1400 IU/kg and assessed clinical adverse effects in addition to laboratory monitoring of serum Ca^{2+} and P levels. Four of the five dogs showed clinical improvement without any adverse reactions. None of the dogs showed laboratory abnormalities. The starting dose was determined due to earlier mice trials[45] and calculations of comparable effects to cholecalciferol in human medicine.

In human beings, there is evidence for an association between atopy and asthma and decreased vitamin D concentrations in blood and skin.[27] Half of the asthmatic children in one study had vitamin D concentrations below the normal range; those concentrations were inversely correlated to prick test responses and positively correlated with the forced expiratory volume.[37] In another study, there was a significant association between the degree of vitamin D deficiency and the severity of AD in children.[27] These studies point to a role of vitamin D in the pathogenesis of atopy. However, while several studies show improvement with vitamin D supplementation,[46–49] sometimes in combination with heliotherapy or vitamin E,[50 51] others have failed to identify clinical improvement.[52]

VDR agonists with less toxicity and strong effects on 1,25-dihydroxycholecalciferol-induced AMP in keratinocytes and other skin cells in vitro have been developed to reduce the risk of vitamin D-related adverse effects such as hypercalcaemia in human beings.[53 54] In human patients, the VDR agonist paricalcitol showed a low potential for hypercalcaemia in immune-mediated diseases with an additional lower risk for adverse effects such as atherosclerosis.[23 55] Other receptor agonists exist, but paricalcitol was chosen for its availability, ease of administration and also for its comparatively low cost, which makes this agent a practical solution in dogs. Despite the good safety profiles in human beings, in this study hypercalcaemia was observed in half of the dogs in the VDR agonist group. Our results indicate that this VDR agonist in dogs probably has a more calcaemic effect than cholecalciferol. Although the total number of dogs treated with paricalcitol is small, the authors conclude that cholecalciferol is preferable to paricalcitol in dogs due to its apparently better safety profile. In addition, vitamin D showed a higher efficacy with respect to the lesions and pruritus of the dogs with AD.

Adverse effects with the placebo were observed in one dog, which developed hypercalcaemia with associated polydipsia and polyuria. In this case, the history and clinical examination were unremarkable and the client refused any further diagnostic work-up, besides the ionised calcium measurement which was mandatory due to the study protocol.

The number of dogs completing all three arms of this cross-over study was very small. The main reason for this lack of compliance was the time-consuming study protocol that required a multitude of visits, particularly in the first two weeks of each arm, leading to a large drop-out during the study after the first and particular the second arm.

A gradual increase in the dose was undertaken, and the final dose of cholecalciferol was frequently not reached until four weeks after inclusion. In future studies, a longer treatment duration could be considered. The increase in serum 25-OH-D3 during the study shows effective absorption of the supplemented cholecalciferol. No adverse clinical effects or serum electrolyte abnormalities were seen with cholecalciferol administration. Additionally, serum-24,25-OH-D3 levels also increased, indicating that the back-up mechanism was activated to metabolise cholecalciferol into the less active form and prevent hypercalcaemia.[56] In three cases, 25-OH-D3- and 24,25-OH-D3-concentrations were measured 16 (n=1) and 20 weeks (n=2) after cessation of therapy, respectively. Serum concentrations were slightly elevated in only one case and in the upper reference range in the residual two cases, still indicating a possible prolonged effect of cholecalciferol, so monitoring for adverse effects may have to be continued beyond vitamin D administration. It is notable that despite this no persistence of clinical efficacy was observed during the eight-week washout period. Possibly, the study period was too short to cause more noticeable/measurable adverse effects. Thus, it is recommended that dogs on vitamin D should be monitored regularly until further studies evaluate long-term safety. The longest follow-up for two dogs treated with cholecalciferol beyond the eight weeks of this study was six months; no clinical adverse effects were noted during that time and hypercalcaemia was not present. In one of these dogs, a 12-week cessation of therapy resulted in disease recurrence with subsequent improvement again on therapy.

It may be possible that 24,25-OH-D3-measurement allows a prediction of long-term adverse effects such as calcium depositions without hypercalcaemia. Although 24,25-OH-D3 concentrations in this study remained elevated for several months in one dog, no adverse effects were noted with administration of cholecalciferol. Longer treatment periods are needed to answer this question. 25-OH-D2 and 3c-E-OH-D3 levels were not changed in this clinical trial.

A sole therapeutic cholecalciferol effect of compensating an increased consumption or even total lack of vitamin D in atopic individuals is possible, but additional effects are presumed. This is supported by an unpublished pilot study conducted by one of the collaborators (HK), suggesting lower vitamin D concentrations in atopic compared with healthy dogs and is suspected in atopic human patients.[26]

Some recent in vitro studies have shown the involvement of AMP in AD in both human beings and dogs.[57 58] In human beings, the synthesis of AMPs is increased under the influence of vitamin D[20] and decreased in vitamin D deficiency.[59] An increase in AMP expression may contribute to the prevention of secondary microbial involvements. In a study recently performed by the authors' group, candidate genes involved in the pathogenesis of canine AD were identified which had not been described previously.[60] BCL3, a transcription factor that modulates AMPs, was upregulated with allergen-mediated inflammation.[60 61] An increase in BCL3 leads to a decrease in AMPs. As vitamin D inhibits the expression of BCL3,[62] this is one possible mechanism for the increase of AMPs and a plausible proposal to explain why vitamin D could be an effective treatment in AD.

TEWL as well as skin pH were measured to attempt to document the postulated improvement of skin barrier function in less lesional atopic dog skin.[63] 1,25-Dihydroxycholecalciferol upregulates genes encoding proteins relevant for the skin barrier function in human beings such as tight junctions, gap junctions and adhesive junctions helping to minimise the penetration of allergens.[64] However, in this study, there was no significant change in TEWL or pH values. Either TEWL and pH were not reliable indicators for the skin barrier function, or the authors were not able to measure these changes in the cutaneous barrier with cholecalciferol at the dose and duration used.

Essential fatty acids are reported to be of benefit in dogs with AD by improving the skin barrier function and reducing inflammatory reactions.[65] Palm oil contains linoleic acid, an essential fatty acid. Cholecalciferol and paricalcitol were both diluted in palm oil for the study blinding, but as there was clinical deterioration in the placebo group, it is unlikely that the results were influenced by the linoleic acid in the placebo.

This study only evaluated a small number of dogs and short-term treatment for eight weeks. However, results suggest that oral vitamin D may be potentially useful for the treatment of canine AD. As dogs are a good animal model for human medicine,[6 7] this should be considered as a promising study for future treatment options in both veterinary and human medicine.

The results of this study suggest that systemic cholecalciferol may be a viable and effective treatment alternative for canine AD as it has been shown for human AD. Further studies will be needed to elucidate the possible beneficial effects of vitamin D on canine AD, evaluate long-term effects and elucidate the mechanisms of cholecalciferol in dogs with AD.

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Competing interests None declared.

References

1 HILLIER A, GRIFFIN CE. The ACVD task force on canine atopic dermatitis (I): incidence and prevalence. *Vet Immunol Immunopathol* 2001;81:147–51.
2 HENSEL P, SANTORO D, FAVROT C, *et al*. Canine atopic dermatitis: detailed guidelines for diagnosis and allergen identification. *BMC Vet Res* 2015;11:196.
3 OLIVRY T, DUNSTON SM, PLUCHINO K, *et al*. Lack of detection of circulating skin-specific IgE autoantibodies in dogs with moderate or severe atopic dermatitis. *Vet Immunol Immunopathol* 2008;122:182–7.
4 HILL PB, DEBOER DJ. The ACVD task force on canine atopic dermatitis (IV): environmental allergens. *Vet Immunol Immunopathol* 2001;81:169–86.
5 SANTORO D, MARSELLA R, BUNICK D, *et al*. Expression and distribution of canine antimicrobial peptides in the skin of healthy and atopic beagles. *Vet Immunol Immunopathol* 2011;144:382–8.
6 ERMEL RW, KOCK M, GRIFFEY SM, *et al*. The atopic dog: a model for food allergy. *Lab Anim Sci* 1997;47:40–9.
7 TEUBER SS, DEL VAL G, MORIGASAKI S, *et al*. The atopic dog as a model of peanut and tree nut food allergy. *J Allergy Clin Immunol* 2002;110:921–7.
8 PUCHEU-HASTON CM, JACKSON HA, OLIVRY T, *et al*. Epicutaneous sensitization with Dermatophagoides farinae induces generalized allergic dermatitis and elevated mite-specific immunoglobulin E levels in a canine model of atopic dermatitis. *Clin Exp Allergy* 2008;38:667–79.
9 YOON JS, NISHIFUJI K, SASAKI A, *et al*. Alteration of stratum corneum ceramide profiles in spontaneous canine model of atopic dermatitis. *Exp Dermatol* 2011;20:732–6.
10 ZUR G, IHRKE PJ, WHITE SD, *et al*. Canine atopic dermatitis: a retrospective study of 266 cases examined at the University of California, Davis, 1992-1998. Part I. Clinical features and allergy testing results. *Vet Dermatol* 2002;13:89–102.
11 OLIVRY T, SOUSA CA. The ACVD task force on canine atopic dermatitis (XIX): general principles of therapy. *Vet Immunol Immunopathol* 2001;81:311–6.
12 NOLI C, MINAFÒ G, GALZERANO M. Quality of life of dogs with skin diseases and their owners. Part 1: development and validation of a questionnaire. *Vet Dermatol* 2011;22:335–43.
13 BEATTIE PE, LEWIS-JONES MS. A comparative study of impairment of quality of life in children with skin disease and children with other chronic childhood diseases. *Br J Dermatol* 2006;155:145–51.
14 OLIVRY T, MUELLER RS. International Task Force on Canine Atopic Dermatitis. Evidence-based veterinary dermatology: a systematic review of the pharmacotherapy of canine atopic dermatitis. *Vet Dermatol* 2003;14:121–46.
15 BLOOM P. Atopic dermatitis in dogs - hitting the moving target. *Vet J* 2006;171:16–17.
16 INMAN AO, OLIVRY T, DUNSTON SM, *et al*. Electron microscopic observations of stratum corneum intercellular lipids in normal and atopic dogs. *Vet Pathol* 2001;38:720–3.
17 DEBOER DJ, MARSELLA R. The ACVD task force on canine atopic dermatitis (XII): the relationship of cutaneous infections to the pathogenesis and clinical course of canine atopic dermatitis. *Vet Immunol Immunopathol* 2001;81:239–49.
18 LEVY S. Reduced bacterial biodiversity is associated with increased allergy. *Environ Health Perspect* 2012;120:a304.
19 HEINE G, HOEFER N, FRANKE A, *et al*. Association of vitamin D receptor gene polymorphisms with severe atopic dermatitis in adults. *Br J Dermatol* 2013;168:855–8.
20 YIM S, DHAWAN P, RAGUNATH C, *et al*. Induction of cathelicidin in normal and CF bronchial epithelial cells by 1,25-dihydroxyvitamin D(3). *J Cyst Fibros* 2007;6:403–10.
21 STRUKOV VI BNA. Side effects and toxic reactions to vitamin D]. *Pediatriia* 1980;3:56–8.
22 JENSEN TJ, HENRIKSEN LO, SØLVSTEN H, *et al*. Inhibition of the 1,25-dihydroxyvitamin D3-induced increase in vitamin D receptor (VDR) levels and binding of VDR-retinoid X receptor (RXR) to a direct repeat (DR)-3 type response element by an RXR-specific ligand in human keratinocyte cultures. *Biochem Pharmacol* 1998:55:767–73.
23 SOCHOROVÁ K, BUDINSKÝ V, ROZKOVÁ D, *et al*. Paricalcitol (19-nor-1,25-dihydroxyvitamin D2) and calcitriol (1,25-dihydroxyvitamin D3) exert potent immunomodulatory effects on dendritic cells and inhibit induction of antigen-specific T cells. *Clin Immunol* 2009;133:69–77.
24 GOLDENBERG MM. Paricalcitol, a new agent for the management of secondary hyperparathyroidism in patients undergoing chronic renal dialysis. *Clin Ther* 1999;21:432–41.
25 VAN DEN OUWELAND JM, BEIJERS AM, DEMACKER PN, *et al*. Measurement of 25-OH-vitamin D in human serum using liquid chromatography tandem-mass spectrometry with comparison to radioimmunoassay and automated immunoassay. *J Chromatogr B Analyt Technol Biomed Life Sci* 2010;878:1163–8.
26 SEARING DA, LEUNG DY. Vitamin D in atopic dermatitis, asthma and allergic diseases. *Immunol Allergy Clin North Am* 2010;30:397–409.
27 PERONI DG, PIACENTINI GL, CAMETTI E, *et al*. Correlation between serum 25-hydroxyvitamin D levels and severity of atopic dermatitis in children. *Br J Dermatol* 2011;164:1078–82.
28 RASSNICK KM, MUINDI JR, JOHNSON CS, *et al*. In vitro and in vivo evaluation of combined calcitriol and cisplatin in dogs with spontaneously occurring tumors. *Cancer Chemother Pharmacol* 2008;62:881–91.
29 REDDY GS, TSERNG KY. Calcitroic acid, end product of renal metabolism of 1,25-dihydroxyvitamin D3 through C-24 oxidation pathway. *Biochemistry* 1989;28:1763–9.
30 NESTEROVA G, MALICDAN MC, YASUDA K, *et al*. 1,25-(OH)2D-24 Hydroxylase (CYP24A1) Deficiency as a Cause of Nephrolithiasis. *Clin J Am Soc Nephrol* 2013;8:649–57.
31 OLIVRY T, MARSELLA R, IWASAKI T, *et al*. International Task Force On Canine Atopic D. Validation of CADESI-03, a severity scale for clinical trials enrolling dogs with atopic dermatitis. *Veterinary Dermatology* 2007;18:78–86.
32 HILL PB, LAU P, RYBNICEK J. Development of an owner-assessed scale to measure the severity of pruritus in dogs. *Vet Dermatol* 2007;18:301–8.
33 CONNELL AB, JENKINS N, BLACK M, *et al*. Overreporting of vitamin D deficiency with the Roche Elecsys Vitamin D3 (25-OH) method. *Pathology* 2011;43:368–71.
34 HØJSKOV CS, HEICKENDORFF L, MØLLER HJ. High-throughput liquid-liquid extraction and LCMSMS assay for determination of circulating 25(OH) vitamin D3 and D2 in the routine clinical laboratory. *Clin Chim Acta* 2010;411:114–6.
35 OLIVRY T, DEBOER DJ, GRIFFIN CE, *et al*. The ACVD task force on canine atopic dermatitis: forewords and lexicon. *Vet Immunol Immunopathol* 2001;81:143–6.
36 PLATTS-MILLS TA. Atopic allergy: asthma and atopic dermatitis. *Curr Opin Immunol* 1991;3:874–80.
37 SEARING DA, ZHANG Y, MURPHY JR, *et al*. Decreased serum vitamin D levels in children with asthma are associated with increased corticosteroid use. *J Allergy Clin Immunol* 2010;125:995–1000.
38 SCHAUBER J, GALLO RL. Antimicrobial peptides and the skin immune defense system. *J Allergy Clin Immunol* 2009;124(3 Suppl 2):R13–18.
39 MARSELLA R, OLIVRY T, CARLOTTI DN. Current evidence of skin barrier dysfunction in human and canine atopic dermatitis. *Vet Dermatol* 2011;22:239–48.
40 WANG TT, NESTEL FP, BOURDEAU V, *et al*. Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. *J Immunol* 2004;173:6490.1–6490.
41 ONG PY, OHTAKE T, BRANDT C, *et al*. Endogenous antimicrobial peptides and skin infections in atopic dermatitis. *N Engl J Med* 2002;347:1151–60.
42 VAN DER AAR AM, SIBIRYAK DS, BAKDASH G, *et al*. Vitamin D3 targets epidermal and dermal dendritic cells for induction of distinct regulatory T cells. *J Allergy Clin Immunol* 2011;127:1532–40.
43 EHLAYEL MS, BENER A, SABBAH A. Is high prevalence of vitamin D deficiency evidence for asthma and allergy risks? *Eur Ann Allergy Clin Immunol* 2011;43:81–8.
44 OREN E, BANERJI A, CAMARGO CA. Vitamin D and atopic disorders in an obese population screened for vitamin D deficiency. *J Allergy Clin Immunol* 2008;121:533–4.
45 RUBEL D, STOCK J, CINER A, *et al*. Antifibrotic, nephroprotective effects of paricalcitol versus calcitriol on top of ACE-inhibitor therapy in the COL4A3 knockout mouse model for progressive renal fibrosis. *Nephrology Dialysis Transplantation* 2014;29:1012–9.
46 CAMARGO CA, GANMAA D, SIDBURY R, *et al*. Randomized trial of vitamin D supplementation for winter-related atopic dermatitis in children. *J Allergy Clin Immunol* 2014;134:831–5.

47 DI FILIPPO P, SCAPARROTTA A, RAPINO D, *et al*. Vitamin D supplementation modulates the immune system and improves atopic dermatitis in children. *Int Arch Allergy Immunol* 2015;166:91–6.

48 UDOMPATAIKUL M, HUAJAI S, CHALERMCHAI T, *et al*. The Effects of Oral Vitamin D Supplement on Atopic Dermatitis: A Clinical Trial with Staphylococcus aureus Colonization Determination. *J Med Assoc Thai* 2015;98(Suppl 9):S23–30.

49 ERKKOLA M, KAILA M, NWARU BI, *et al*. Maternal vitamin D intake during pregnancy is inversely associated with asthma and allergic rhinitis in 5-year-old children. *Clin Exp Allergy* 2009;39:875–82.

50 JAVANBAKHT MH, KESHAVARZ SA, DJALALI M, *et al*. Randomized controlled trial using vitamins E and D supplementation in atopic dermatitis. *J Dermatolog Treat* 2011;22:144–50.

51 VÄHÄVIHU K, YLIANTTILA L, SALMELIN R, *et al*. Heliotherapy improves vitamin D balance and atopic dermatitis. *Br J Dermatol* 2008;158:1323–8.

52 FEILY A, NAMAZI MR. Vitamin A + D ointment is not an appropriate emollient for atopic dermatitis. *Dermatitis* 2010;21:174–5.

53 MA Y, KHALIFA B, YEE YK, *et al*. Identification and characterization of noncalcemic, tissue-selective, nonsecosteroidal vitamin D receptor modulators. *J Clin Invest* 2006;116:892–904.

54 HARTMANN B. Vitamin D receptor activation modulates the allergic immune response. Berlin Charité: Berlin Charité; dissertation, 2011.

55 BALINT E, MARSHALL CF, SPRAGUE SM. Effect of the vitamin D analogues paricalcitol and calcitriol on bone mineral in vitro. *Am J Kidney Dis* 2000;36:789–96.

56 VELDURTHY V, WEI R, CAMPBELL M, *et al*. 25-Hydroxyvitamin D(3) 24-Hydroxylase: a key regulator of 1,25(OH)(2)D(3) Catabolism and Calcium Homeostasis. *Vitamines and Hormones* 2016;100:137–50.

57 SANTORO D, AHRENS K, MARSELLA R, *et al*. Evaluation of antimicrobial peptides and cytokine production in primary keratinocyte cell culture from healthy and atopic beagles. *Exp Dermatol* 2015;24:317–9.

58 HOWELL MD, NOVAK N, BIEBER T, *et al*. Interleukin-10 downregulates anti-microbial peptide expression in atopic dermatitis. *J Invest Dermatol* 2005;125:738–45.

59 KIM SK, PARK S, LEE ES. Toll-like receptors and antimicrobial peptides expressions of psoriasis: correlation with serum vitamin D level. *J Korean Med Sci* 2010;25:1506–12.

60 SCHAMBER P, SCHWAB-RICHARDS R, BAUERSACHS S, *et al*. Gene Expression in the Skin of Dogs Sensitized to the House Dust Mite Dermatophagoides farinae. G3: Genes, Genomes. *Genetics* 2014;4:1787–95.

61 BÜCHAU AS, MACLEOD DT, MORIZANE S, *et al*. Bcl-3 acts as an innate immune modulator by controlling antimicrobial responses in keratinocytes. *J Invest Dermatol* 2009;129:2148–55.

62 FUKUYA Y, HIGAKI M, HIGAKI Y, *et al*. Effect of vitamin D3 on the increased expression of Bcl-xL in psoriasis. *Arch Dermatol Res* 2002;293:620–5.

63 OLIVRY T. Is the skin barrier abnormal in dogs with atopic dermatitis? *Vet Immunol Immunopathol* 2011;144:11–16.

64 BIKLE DD, CHANG S, CRUMRINE D, *et al*. 25 Hydroxyvitamin D 1 alpha-hydroxylase is required for optimal epidermal differentiation and permeability barrier homeostasis. *J Invest Dermatol* 2004;122:984–92.

65 BENSIGNOR E, MORGAN DM, NUTTALL T. Efficacy of an essential fatty acid-enriched diet in managing canine atopic dermatitis: a randomized, single-blinded, cross-over study. *Vet Dermatol* 2008;19:156–62.

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