

Management of canine atopic dermatitis using the plant extract PYM00217: a randomized, double-blind, placebo-controlled clinical study

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This study was funded by Phytopharm plc., Godmanchester, UK.

Some of the data were presented in abstract form at the 5th World

Congress in Veterinary Dermatology, Vienna, 26–28 August 2004.

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PYM00217 at 200 mg kg⁻¹ appears to be an effective, palatable and well-tolerated treatment for canine AD.

Accepted 08 April 2006

Abstract

This study evaluated PYM00217, a proprietary blend of plant extracts, in the management of canine atopic dermatitis (AD). One hundred and twenty dogs were diagnosed with perennial AD on the basis of history, clinical signs, a positive test for perennial allergens and elimination of other dermatoses. Exclusion criteria included antimicrobials within 7 days, antihistamines within 14 days, oral/topical glucocorticoids or ciclosporin within 28 days, and parenteral glucocorticoids, essential fatty acids or immunotherapy within 56 days. Flea control, shampoos and ear cleaners were permitted. Dogs with a minimum canine atopic dermatitis extent and severity index (CADESI) of 25 were randomly allocated to receive PYM00217 (100, 200 or 400 mg kg⁻¹ day⁻¹) or placebo for 12 weeks. The mean reductions in CADESI (intention-to-treat population) were 3.9% (placebo; $n = 29$), 4.4% (100 mg kg⁻¹ day⁻¹; $n = 30$), 23.4% (200 mg kg⁻¹ day⁻¹; $n = 29$) and 8.5% (400 mg kg⁻¹ day⁻¹; $n = 29$). The reduction in the 200 mg kg⁻¹ day⁻¹ group was significant ($P < 0.01$). For dogs with a baseline CADESI ≥ 50 , the mean changes were +10.6% (placebo; $n = 12$), +0.6% (100 mg kg⁻¹ day⁻¹; $n = 14$), -29.3% (200 mg kg⁻¹ day⁻¹; $n = 14$) and -3.4% (400 mg kg⁻¹ day⁻¹; $n = 15$). The 200 mg kg⁻¹ day⁻¹ dose was significantly more effective than placebo ($P = 0.038$). No serious adverse effects were reported. Minor adverse effects seen in 10% (placebo and 100 mg kg⁻¹ day⁻¹), 24% (200 mg kg⁻¹ day⁻¹) and 42% (400 mg kg⁻¹ day⁻¹) of cases were mainly minor gastrointestinal disorders and only five cases required cessation of dosing. Two dogs (one in each of the 100 mg kg⁻¹ day⁻¹ and 200 mg kg⁻¹ day⁻¹ groups) refused to eat the medicated food. In conclusion,

Introduction

Canine atopic dermatitis (AD) is a common condition estimated to affect 10–15% of the canine population.^{1–4} The complex pathomechanism and interactions underlying pruritus make AD a challenging disease to manage. Many atopic dogs require long-term anti-inflammatory medication, but a recent evidence-based review⁵ concluded that there was good evidence to support the use of only oral glucocorticoids and ciclosporin. Fair evidence exists for the use of tacrolimus lotion, triamcinolone spray, oral pentoxifylline and misoprostol. Insufficient evidence exists either for or against the use of any other treatments, currently including Chinese herbal therapy, with evidence existing against the use of arofylline, and leukotriene synthesis inhibitors and receptor antagonists. Adverse effects have been reported in 30–80% of animals receiving glucocorticoids and 14–81% of those receiving ciclosporin.⁵ Owners are frequently unwilling to accept even minor adverse effects, while serious adverse effects would be unacceptable on ethical and medical grounds given the non-life threatening nature of canine AD. There thus remains an unmet need for safe and effective alternative therapies.

Preparations based on traditional Chinese medicine have been shown in double-blind, placebo-controlled cross-over studies to be beneficial in the management of intractable human AD.^{6–8} The preparations used for these studies consisted of an extract of 10 Chinese medicinal plants (Zemaphyte™; Phytopharm plc, Godmanchester, UK) that inhibits interleukin (IL)-4-mediated induction of the low-affinity immunoglobulin (Ig) E receptor (CD23) on mononuclear cells.⁹ In a subsequent veterinary study, P07P (an extract obtained from three of the original 10 plants, *Rehmannia glutinosa*, *Paeonia lactiflora* and *Glycyrrhiza uralensis*, which were selected on the basis of *in vitro* bioassay data and palatability) reduced erythema and pruritus in canine AD.¹⁰ No adverse effects were seen with the exception of mild diarrhoea and flatulence in a small minority of dogs (five of 24 cases).

The product that was used for this study, PYM00217, was manufactured from the same plants and supplied by Phytopharm plc. The aim of this study was to further evaluate the safety, efficacy and dose-response profile of

PYM00217, by using a randomized, double-blind, placebo-controlled, parallel group design, large-scale study.

Materials and methods

Estimation of group size and treatment allocation

It was estimated that there would be a mean reduction in the canine atopic dermatitis extent and severity index (CADESI) score in the placebo group of 8% with a standard deviation of 18% (T. Olivry, pers. comm.). Assuming a 13% (as suggested by previous open-label and controlled studies) greater reduction in response to dosing with PYM00217, 30 subjects in each group would be required to detect a statistically significant difference in response between the groups with 80% power. A total of 120 dogs would therefore be required to permit four dose groups of 30 animals. Eleven UK and three French dermatology referral centres participated. One hundred and twenty dogs fulfilling the entry criteria (Table 1) were randomly allocated between four groups: PYM00217 (100 mg kg⁻¹ day⁻¹), PYM00217 (200 mg kg⁻¹ day⁻¹), PYM00217 (400 mg kg⁻¹ day⁻¹) and placebo. The clinical supplies were independently allocated and labelled according to a computer generated master randomization code with a 1 : 1 : 1 : 1 group ratio (DHP Ltd, Abergavenny, UK).

The PYM00217 and placebo products were supplied as granules of similar colour and size, packed in identical sealed aluminium sachets and distinguished only by a pack number, so that the owners and investigators were blinded to the sachet contents. Owners were instructed to add the appropriate quantity of granules to their dog's food once daily. All unused and empty sachets were retained and returned to the clinic at each visit to allow the assessment of owner compliance. The proportion of dosed food eaten each day was recorded by the owners in a daily diary to assess subject compliance.

The study protocol was approved by the Ethics Committee of the Royal Veterinary College and carried out under the terms of an animal test certificate (ATC No. 12674/0003) granted by the Veterinary Medicines Directorate, Department of Food, the Environment and Rural Affairs (DEFRA), UK.

Inclusion and exclusion criteria

Inclusion and exclusion criteria are listed in Table 1. A diagnosis of AD was made using currently accepted criteria and by the exclusion of other

pruritic diseases.^{11,12} Dogs with superficial pyoderma or *Malassezia pachydermatis* associated dermatitis were treated appropriately until clinical signs had resolved. Dogs were stabilized on their usual diet for at least 2 weeks prior to study enrolment and owners were instructed not to change the diet offered during the study.

Clinical assessment

The clinical severity was assessed using a modified CADESI score.¹³ This involved a subjective (normal = 0, mild = 1, moderate = 2 and severe = 3) assessment of erythema, lichenification and excoriation at 40 body sites to give a total score of 0–360. The minimum CADESI score at entry was 25. Details of demographics, medical history, treatments received for AD within the previous 6 months, current treatment for AD and concomitant medications were recorded.

At weeks 0, 4, 8 and 12, a full clinical examination was performed. The same investigator examined a particular dog throughout the study. The investigator recorded the CADESI score and made an assessment of the overall response in comparison with the baseline pre-dose state (markedly worse, worse, no change, improved or markedly improved). Owners were asked to assess the severity of pruritus (Table 2) and make an assessment of the overall response as above. Any changes in concomitant medication or adverse events (AEs) were noted. Dogs were withdrawn if they required treatment with a prohibited medication (Table 1), for poor compliance or if they showed unacceptable discomfort. End of dosing assessments were then recorded. Owners were free to withdraw their animals at any point. Participation was concluded by a follow-up safety assessment by telephone 2 weeks after cessation of dosing.

The primary outcome measure was the total CADESI score¹³ at the end of treatment. Secondary outcome measures included the owners' assessment of pruritus and overall response assessed by the owners and investigators. The outcome for the subgroup of dogs with more severe disease that had a baseline CADESI ≥ 50 was also analysed. The intention-to-treat population was used for analysis. This included all subjects who provided on-treatment data for the duration of the 12-week study or up to the last data recorded prior to withdrawal. This corresponds to a last-observation-carried-forward approach. Three of the 120 subjects (one in the 100 mg kg⁻¹ day⁻¹ group and two in the 400 mg kg⁻¹ day⁻¹ group) did not have any on-treatment assessment and were excluded from the inferential analysis. There were otherwise no violations to randomization, treatment assignment or adherence.

Table 1. Study entry criteria

Inclusion criteria

1. Dog is 18 months of age or older and up to 50 kg in weight
2. Clinical diagnosis of atopic dermatitis^{11,12}
3. A positive intradermal allergen test or allergen-specific IgE ELISA (Allercept®, Heska, Fort Collins, CO, USA) to one or more nonseasonal allergens
4. Persistence of pruritus following a 6-week novel (home cooked or commercial) or hydrolysed exclusion diet
5. History of perennial pruritus and a modified CADESI¹³ ≥ 25
6. No response to a veterinary approved flea-control regimen for at least 8 weeks and monthly flea control throughout the trial period
7. The dog's environment has been treated with a veterinary approved flea elimination programme for at least 2 months
8. Sarcoptic mange excluded by trial therapy and/or negative serology
9. Immunotherapy permitted if in use for > 12 months, the dose remained unchanged for 6 months, the clinical signs were stable and the dosing regimen was not altered during the trial
10. Essential fatty acids were permitted if in use for > 8 weeks, the clinical signs were stable and the dosing regimen was not altered during the trial
11. Antimicrobial or other shampoos and ear cleaners were permitted if in use for at least 3 weeks prior to and the frequency of use did not change during the trial
12. Owners written informed consent has been obtained

Exclusion criteria

1. Clinical evidence of ectoparasite infestation
2. Clinical evidence of bacterial or fungal cutaneous infection
3. Has received antimicrobial therapy or prostaglandins within 7 days
4. Has received antihistamines within 14 days
5. Has received oral or topical glucocorticoids or ciclosporin within 28 days
6. Has received parenteral depot glucocorticoids within 56 days
7. Has initiated or discontinued essential fatty acids within 56 days
8. Has had allergen-specific immunotherapy discontinued within 6 months or initiated within 12 months
9. Pregnancy or breeding activity
10. Has a concurrent medical condition that may deteriorate during the study

Table 2. Scoring system for the owners' assessment of pruritus

Score	Category	Description
0	No itching	Scratching, rubbing, chewing or licking is within normal limits
1	Mild itching	Scratching, rubbing, chewing or licking more than normal, but not affecting daily activities such as eating, playing and exercising
2	Mild to moderate itching	Scratching, rubbing, chewing or licking quite often, but not generally affecting daily activities
3	Moderate itching	Scratching, rubbing, chewing or licking that generally affects daily activities
4	Moderate to severe itching	Scratching, rubbing, chewing or licking that severely affects daily activities and/or disturbs the normal sleep pattern of the dog
5	Severe itching	Scratching, rubbing, chewing or licking throughout the day and/or prevents the dog from sleeping at night

Statistical analysis

A one-way analysis of covariance (ANCOVA) with baseline as the covariate was used to compare the change in CADESI, pruritus and overall response scores between the treatment groups at the end of the treatment period. Use of baseline as a covariate allows comparisons between treatment groups adjusted for any baseline differences and also a more precise comparison between groups. Pairwise comparisons were then made between each dose group and the placebo group. Paired *t*-tests were used for within group comparisons of on-treatment assessments with baseline. The proportion of dogs in each group that achieved a greater than 50% reduction in CADESI and that had a final assessment of either improved or markedly improved was summarized using frequency tables and the groups were compared using Fisher's exact tests. The level of statistical significance was set at $P < 0.05$ (two-sided).

Results

Subject demographics

The number of dogs starting and finishing the trial, breed distribution, mean age, sex, bodyweight, baseline CADESI and baseline pruritus scores, and use of concomitant medications were similar between groups (Table 3).

Clinical assessment (CADESI)

For the intention-to-treat population, a comparison of the change from baseline between groups did not confirm a statistically significant treatment effect (ANCOVA, $P = 0.30$). Dogs that were treated with PYM00217 (200 mg kg⁻¹ day⁻¹), however, did experience a statistically significant reduction in their mean CADESI at the end of treatment assessment compared to their mean baseline score (paired *t*-test, $P < 0.01$) (Table 4a and Fig. 1a). By contrast, no significant change in CADESI was detected for any of the other groups.

The effect of treatment with PYM00217 (200 mg kg⁻¹ day⁻¹) was particularly apparent in the subgroup of dogs with more severe disease (i.e. baseline CADESI ≥ 50) (Table 4b and Fig. 1a). Further analysis of the between-group change from baseline CADESI for these animals demonstrated a statistically significant difference between the placebo (10.6% increase) and PYM00217 (200 mg kg⁻¹ day⁻¹; 29.3% reduction) dose groups (unpaired *t*-test, $P = 0.038$).

The primary outcome measure of the study was the change in CADESI at the end of the study compared to baseline. These data are summarized for all dogs and the baseline CADESI ≥ 50 subgroup in Fig. 1(a). It is interesting to note that the changes in CADESI at the end of treatment were similar to those seen after 4 weeks treatment (Table 4c) in both groups. Repeated measures and time-point analysis were not a priori-established outcome measures and were not undertaken.

A larger proportion of dogs in the PYM00217 (200 mg kg⁻¹ day⁻¹) dose group gained a more than 50% reduction in their baseline CADESI compared with the other treatment and placebo groups (Table 4a,b, and Fig. 1b), although the differences between groups were not significant.

Overall response

Both owners and investigators reported that a substantial proportion of dogs in the PYM00217 (200 and 400 mg kg⁻¹ day⁻¹) and placebo groups had either improved or markedly improved (Table 5). Interestingly, when dogs with a baseline CADESI ≥ 50 were analysed, both the owners and the investigators considered that dogs that received PYM00217 (200 mg kg⁻¹ day⁻¹) responded better than the dogs in the other treatment groups ($P < 0.05$).

Table 3. Subject demographics and concomitant medication

	100 mg kg ⁻¹ day ⁻¹	200 mg kg ⁻¹ day ⁻¹	400 mg kg ⁻¹ day ⁻¹	Placebo
No. of dogs starting trial	31	29	31	29
No. of dogs withdrawn	7	8	11	8
No. of dogs with intention to treat data	30	29	29	29
Age (years); mean (SD)	5.38 (3.0)	5.44 (2.35)	5.31 (2.43)	5.27 (2.9)
Body weight (kg); mean (SD)	19.63 (11.04)	22.24 (13.0)	20.32 (12.08)	21.24 (11.47)
% Male	48	55	52	55
% Female	52	45	48	45
Concomitant medication:				
Topical antimicrobials	14	15	15	15
Other topical agents	12	12	11	10
Ear cleaners	13	8	9	12
Allergen-specific immunotherapy	4	6	7	6
Essential fatty acids	6	6	6	7

Table 4. CADESI before and after treatment with PYM00217 or placebo once daily for up to 12 weeks

a) All dogs (intention-to-treat population)

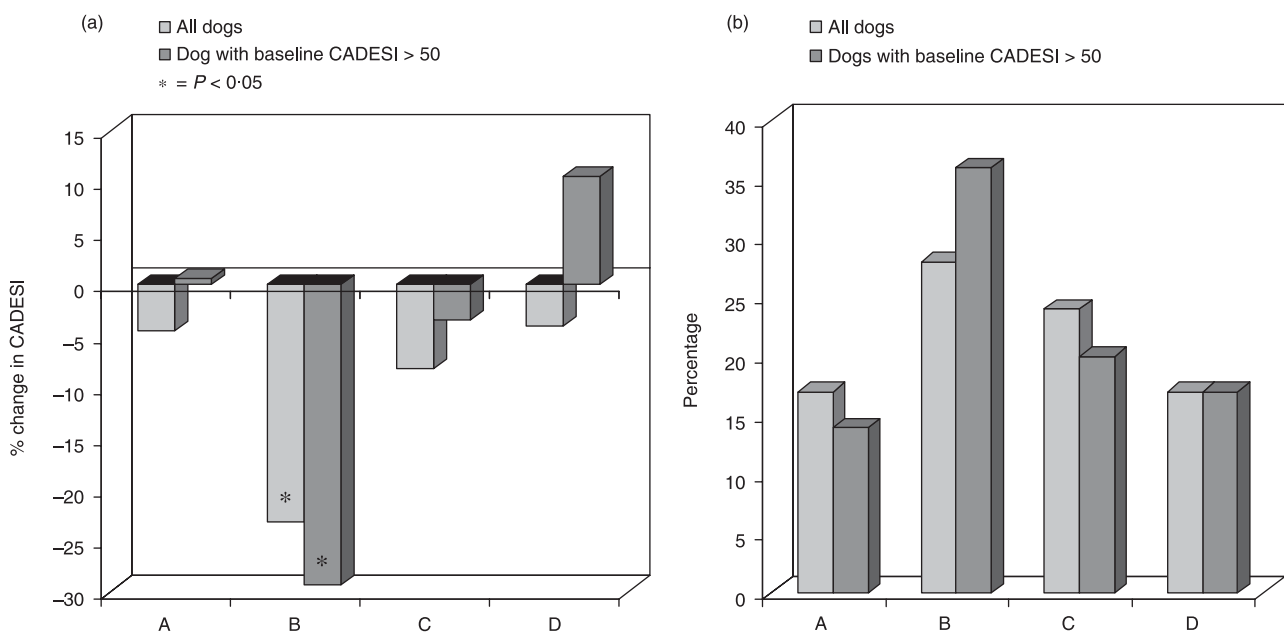
Mean CADESI (SD, <i>n</i>)	100 mg kg ⁻¹ day ⁻¹	200 mg kg ⁻¹ day ⁻¹	400 mg kg ⁻¹ day ⁻¹	Placebo
Baseline	49.7 (18.27, 30)	58.0 (24.58, 29)	57.6 (26.2, 29)	46.7 (15.79, 29)
End of treatment	47.5 (33.04, 30)	44.4 (28.46, 29)	52.7 (38.06, 29)	44.9 (32.89, 29)
No. of dogs with a = 50% reduction in CADESI	5	8	7	5

b) Dogs with baseline CADESI = 50

Mean CADESI (SD, <i>n</i>)	100 mg kg ⁻¹ day ⁻¹	200 mg kg ⁻¹ day ⁻¹	400 mg kg ⁻¹ day ⁻¹	Placebo
Baseline	67.3 (8.56, 14)	79.4 (17.06, 14)	77.1 (24.07, 15)	63.3 (9.63, 12)
End of treatment	67.6 (34.55, 14)	56.1 (32.47, 14)	74.5 (38.93, 15)	69.9 (36.24, 12)
No. of dogs with a = 50% reduction in CADESI	2	5	3	2

c) Mean change in baseline CADESI (%) seen after treatment with PYM00217 or placebo once daily for 4 weeks

CADESI – mean change from baseline (%)	100 mg kg ⁻¹ day ⁻¹	200 mg kg ⁻¹ day ⁻¹	400 mg kg ⁻¹ day ⁻¹	Placebo
All dogs	-5.9	-26	-8.6	+1.5
Dogs with baseline CADESI = 50	-2.8	-32.2	-6.0	+15.5

**Figure 1.** (a) Reduction in mean CADESI at the end of treatment compared to baseline in dogs treated with: A – PYM00217 100 mg kg⁻¹; B – 200 mg kg⁻¹; C – 400 mg kg⁻¹; or D – placebo once daily for up to 12 weeks. (b) Proportion of dogs that achieved a greater than 50% reduction in CADESI at the end of treatment compared to baseline following treatment with: A – PYM00217 100 mg kg⁻¹; B – 200 mg kg⁻¹; C – 400 mg kg⁻¹; or D – placebo once daily for up to 12 weeks.

Changes in the owners' perception of pruritus

The owners' perception of pruritus severity scores declined during the study (Table 6), although this was most marked for the PYM00217 (400 mg kg⁻¹ day⁻¹) group. Dogs with a baseline CADESI $\geq$ 50 that received PYM00217 (200 and 400 mg kg⁻¹ day⁻¹) had a greater reduction in pruritus than either the PYM00217 (100 mg kg⁻¹ day⁻¹) or the placebo groups. There were, however, no statistically significant differences in pruritus scores between or within any of the dose groups (ANCOVA, $P > 0.05$).

Safety profile

There were no serious AEs attributable to either PYM00217 or the placebo. At least one AE that was possibly attributable to study drug was experienced by 10% (placebo), 10% (100 mg kg⁻¹ day⁻¹), 24% (200 mg kg⁻¹ day⁻¹) and 42% (400 mg kg⁻¹ day⁻¹) of dogs. These were typically intermittent, self-

limiting gastrointestinal disorders that prompted cessation of therapy in only five dogs.

Thirty-four dogs were prematurely withdrawn from the study. Five (placebo), five (100 mg kg⁻¹ day⁻¹), three (200 mg kg⁻¹ day⁻¹) and six (400 mg kg⁻¹ day⁻¹) dogs were withdrawn because their clinical signs required treatment with prohibited medications. Five dogs were withdrawn due to AEs; two (vomiting; flatulence), one (diarrhoea) and two (diarrhoea; diarrhoea and vomiting) cases in the placebo, PYM00217 at 200 mg kg⁻¹ day⁻¹ and at 400 mg kg⁻¹ day⁻¹ dose groups, respectively. Two dogs were withdrawn due to intercurrent illnesses (hepatocellular adenoma [100 mg kg⁻¹ day⁻¹] and malignant lymphoma [200 mg kg⁻¹ day⁻¹]) that were unlikely to have been attributable to PYM00217. Two dogs (400 mg kg⁻¹ day⁻¹) were lost to follow-up and owner consent was withdrawn for one dog in each of the placebo and 400 mg kg⁻¹ day⁻¹ dose groups. Just one dog in each of the

Table 5. Owners and investigators opinion of overall improvement at the end of treatment compared to baseline in dogs treated with PYM00217 or placebo once daily for up to 12 weeks ($P < 0.05$ compared to other treatment groups)

Treatment group	Cases improved or markedly improved (%)			
	Owners' impression		Investigators' impression	
	All dogs	Dogs with a baseline CADESI = 50	All dogs	Dogs with a baseline CADESI = 50
100 mg kg ⁻¹ day ⁻¹	27.0	36.0	30.0	29.0
200 mg kg ⁻¹ day ⁻¹	45.0	50.0	38.0	43.0
400 mg kg ⁻¹ day ⁻¹	45.0	40.0	45.0	33.0
Placebo	45.0	42.0	38.0	25.0

Table 6. Changes in the owners' perception of pruritus (defined in Table 2) at the end of treatment compared to baseline in dogs treated with PYM00217 or placebo once daily for up to 12 weeks

a) All dogs

Mean pruritus score (SD, <i>n</i>)	100 mg kg ⁻¹ day ⁻¹	200 mg kg ⁻¹ day ⁻¹	400 mg kg ⁻¹ day ⁻¹	Placebo
Baseline	2.87 (1.31, 31)	2.69 (1.23, 29)	2.87 (1.24, 30)	2.59 (1.02, 29)
End of treatment	2.65 (1.39, 31)	2.40 (1.36, 29)	2.26 (1.42, 30)	2.23 (1.37, 29)
Reduction from baseline (%)	7.7	10.8	21.3	13.9

b) Dogs with a CADESI = 50 at baseline

Mean pruritus score (SD, <i>n</i>)	100 mg kg ⁻¹ day ⁻¹	200 mg kg ⁻¹ day ⁻¹	400 mg kg ⁻¹ day ⁻¹	Placebo
Baseline	3.00 (1.11, 14)	2.93 (1.14, 14)	3.13 (1.20, 16)	2.67 (1.16, 12)
End of treatment	2.81 (1.38, 14)	2.35 (1.03, 14)	2.24 (1.47, 16)	2.25 (1.64, 12)
Reduction from baseline (%)	6.3	19.8	28.4	15.7

PYM00217 100 mg kg⁻¹ day⁻¹ and 200 mg kg⁻¹ day⁻¹ groups refused to eat the medicated food. The daily diary completed by the owners indicated that at least 93% of the dosed meals were eaten by the animals in all groups.

Discussion

This study reports the safety, tolerability and efficacy of the plant-derived product PYM00217 in the treatment of canine AD. The optimum dosing regimen appears to be 200 mg kg⁻¹ day⁻¹. At this dose, the mean reduction in CADESI was 23.4% and eight of 29 dogs experienced a > 50% reduction in CADESI. The effect in the more severely affected dogs was more marked: the mean reduction in CADESI was 29.3%, with a > 50% reduction in five of 14 dogs. The findings in this study are in agreement with those from a previous, smaller study that also demonstrated a favourable (albeit not statistically significant) response to P07P (200 mg kg⁻¹ day⁻¹), a product with the same active ingredients as PYM00217.¹⁰

The efficacy demonstrated by this study is at least as good as, and in many cases superior to, that reported for other systemic steroid-sparing agents that are administered to dogs with AD, such as antihistamines, pentoxifylline, arofylline, leukotriene inhibitors and misoprostol.⁵ Most of the adverse effects that were considered to be possibly attributable to PYM00217 were self-limiting gastrointestinal disturbances such as diarrhoea and vomiting. These appeared to be dose-related and were seen in 10% of the placebo, 10% of the 100 mg kg⁻¹ day⁻¹, 24% of the 200 mg kg⁻¹ day⁻¹ and 42% of the 400 mg kg⁻¹ day⁻¹ groups. In only five dogs (two placebo, one 200 mg kg⁻¹ day⁻¹ and two 400 mg kg⁻¹ day⁻¹), however, were these of a severity that necessitated withdrawal of medication. This is generally a better safety profile than has been reported for antihistamines,

pentoxifylline, arofylline, leukotriene inhibitors, misoprostol, ciclosporin and, in particular, glucocorticoids.¹⁴

A bell-shaped dose-response curve to PYM00217 was demonstrated by this study. The reduced effect observed in the highest dose group (400 mg kg⁻¹ day⁻¹) may have been a consequence of the diarrhoea that was more frequent in this group. It is also known that bell-shaped dose-response curves are common in *in vitro* immunological models such as canine peripheral blood mononuclear cell (PBMC) cultures.¹⁵ PYM00217 is a standardized mixture of extracts obtained from three plants (*R. glutinosa*, *P. lactiflora* and *G. uralensis*) and each extract is likely to contain a number of active moieties. The dose-response relationship may therefore depend on the complex interaction of the moieties with each other and the recipient's immune system. *In vitro* and rodent models exposed to these extracts have demonstrated a number of immunomodulating effects. *Glycyrrhiza uralensis* potentiates the expression of the immunosuppressive cytokine IL-10 by human PBMCs, inhibits cytochrome P450, induces apoptosis in leukaemic cells, and has antioxidant and antibacterial activity.¹⁶⁻²¹ *Rehmannia glutinosa* inhibits production of the proinflammatory cytokines (e.g. tumour necrosis factor- α and IL-1), suppresses histamine release and cyclooxygenase activity in rodent cell models, and behaves as an antioxidant *in vitro*.^{16,22,23} *Paeonia lactiflora* inhibits cyclooxygenase activity in leucocytes and nitric oxide synthase in activated macrophages, induces apoptosis in leukaemic cell lines and has antioxidant activity.^{16,24-27} Interestingly, the plasma concentration of paeoniflorin (a known component of *P. lactiflora* extract) obtained after a fixed oral dose in a rodent model exhibits a bell-shaped curve with respect to the co-administered dose of *G. uralensis* extract, so that maximal absorption of paeoniflorin is achieved at a dose of *G. uralensis* equivalent to that present in PYM00217 (200 mg kg⁻¹) (data on file; Phytopharm plc).

It is interesting to note that the response to PYM00217 (200 mg kg⁻¹ day⁻¹) was greater in the more severely affected dogs (i.e. those with a baseline CADESI $\geq$ 50) for all the study outcome measures. With hindsight, it is likely that the minimum CADESI of 25 required for study enrolment was too low. Experienced clinicians are likely to detect minor clinical signs that may have little clinical significance but are associated with a CADESI score that exceeds this threshold. It may be difficult therefore for an effective treatment protocol to achieve a substantial reduction in the clinical signs of these cases. The response to treatments used during the pre-study period may also have biased the owner and investigator assessment of the response observed during the study. Any (even subconscious) comparison of a dog's condition at the end of treatment visit to that at the time of referral rather than study enrolment could therefore have overestimated the placebo effect. This is, however, likely to have had less impact in the more severely affected dogs.

Atopic dermatitis is a multifactorial disease and the condition of many dogs will improve following dietary changes, parasite control, elimination of secondary infections and nonspecific topical therapy, etc.^{3,4,28} A 12-week trial period therefore is suitable for the evaluation of this chronic, relapsing condition. Shorter trials could suffer from considerable carry-over and placebo effects. Interestingly, the end of treatment results was similar to the mean change in CADESI recorded after 4 weeks treatment in both groups of dogs. This suggests that a response to PYM00217 may be seen after 4 weeks, although further study and time-point analyses would be necessary to confirm this.

The CADESI was used as one of the primary outcome measures for this study. It was developed in 1997²⁹ to provide an objective measure of acute inflammation (erythema), chronic inflammation (lichenification) and pruritus (excoriation). Modified indices have since been used as outcome measures for a number of clinical trials.⁵ One study reported that the CADESI had high intra-observer reliability and was a relevant and reliable assessment of clinical severity.¹³ The CADESI scores obtained during the present trial were clearly more discriminatory than the subjective assessments that were made of the overall response and change in severity of pruritus.

The subjective assessments of the overall response provided by both the owners and the investigators correlated with the CADESI ($P < 0.01$; data not presented). The categorical scale used, however, consisted of only five stages and positive grades were limited to 'improved' or 'markedly improved'. Unlike a CADESI score, this provides little indication of the degree of improvement. The change in CADESI is, therefore, more likely to provide a reliable and accurate measure of therapeutic benefit as distinct from a marginal placebo effect.

The pruritus score declined in all the treatment groups during the study. The greatest decrease was seen in the PYM00217 (400 mg kg⁻¹ day⁻¹) group, but there were no statistically significant differences between the end of treatment and baseline assessments in any of the treatment groups. There are a number of possible reasons for the discrepancy between the changes in CADESI and pruritus scores. The use of a limited ordinal score with rigid grade descriptors may not be sufficiently discriminating to detect

an effect. Rigid grade descriptors, furthermore, may be confusing for owners whose animals' behaviour spans more than one grade. Visual analogue scales may be more useful but are still subjective. It is also possible that the change in CADESI score does not correlate with the change in pruritus. The total CADESI includes excoriation as a measure of self-trauma, but despite this it is predominantly a dermatological assessment of cutaneous inflammation and trauma. This can be more reliably and objectively assessed, but may not be as relevant to owners and, therefore, as good a measure of quality of life as pruritus. An alternative explanation for the discrepant results of this study is that PYM00217 could have differential effects on inflammation and pruritus as a consequence of its complex pharmacology. The optimum dose for any one individual could therefore be a balance of the anti-inflammatory and antipruritic activities.

Concomitant medication scores^{30,31} were not used. Concomitant medication in this study was limited in scope, had a minimum pre-trial stabilization period, had to be constant throughout the trial and was similar between all four treatment groups. Any animal that required further therapy during the trial was, furthermore, withdrawn. It is therefore unlikely that medication scores would have added much to the outcome measures already discussed.

This trial was conducted according to good clinical practice and meets the standard for grade A quality.⁵ It was randomized, double blind and placebo controlled with stringent entry criteria. Demographic data and concomitant therapies, furthermore, were comparable between all the groups. It is, therefore, unlikely that selection, inclusion, performance, reporting or detection bias could have influenced the results.⁵ Intention-to-treat analyses included data from subjects prematurely withdrawn, exclusion of which would otherwise have resulted in an artificially high estimation of efficacy.⁵ Multicentre trials can suffer from investigator bias. This, however, was minimized by the use of stringent and objective criteria for inclusion, exclusion and withdrawal from treatment, and stringent and (where possible) objective assessment criteria. In addition, individual dogs were assessed by the same investigator and analyses were restricted to the change from baseline at the end of treatment.

Acknowledgements

The authors wish to acknowledge the support of Ms. M. Cawood (The University of Liverpool Faculty of Veterinary Science) and Dr A. Kelly (study co-ordinator, Phytopharm plc) in the conduct of this trial and preparation of the manuscript. They are also grateful to Mr M. Stevens (Emstat Ltd) for his expert statistical support and advice. This study was funded by Phytopharm plc., Godmanchester, UK. Dr R. Grover was an employee of Phytopharm plc at the time of conducting the study.

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Résumé Cette étude a évalué le PYM00217, un mélange d'extraits de plantes, pour le traitement de la dermatite atopique du chien (AD). Cent vingt chiens ont été diagnostiqués comme souffrant d'AD sur la base de l'anamnèse, de l'examen clinique, d'un test d'allergie positif et de l'exclusion des autres causes de prurit. Les critères d'exclusion étaient l'administration d'antimicrobiens depuis moins de 7 jours, d'antihistaminiques depuis moins de 14 jours, de glucocorticoïdes ou de ciclosporine depuis moins de 28 jours et de glucocorticoïdes injectables, d'acides gras essentiels ou d'une immunothérapie depuis moins de 56 jours. Un traitement insecticide, des shampooings et les nettoyants auriculaires étaient autorisés. Les chiens présentant un score CADESI minimum de 25 ont été traités au hasard avec le PYM00217 (100, 200 ou 400 mg/kg/jour) ou avec

un placebo pendant 12 semaines. La réduction moyenne du CADESI (intention-to-treat population) était de 3.9% (placebo; $n = 29$), 4.4% (100 mg/kg/jour; $n = 30$), 23.4% (200 mg/kg/jour; $n = 29$) et 8.5% (400 mg/kg/jour; $n = 29$). La diminution dans le groupe 200 mg/kg/jour était significative ($P < 0.01$). Pour les chiens avec un CADESI basal ≥ 50 les modifications moyennes étaient +10.6% (placebo; $n = 12$), +0.6% (100 mg/kg/jour; $n = 14$), -29.3% (200 mg/kg/jour; $n = 14$) et -3.4% (400 mg/kg/jour; $n = 15$). La dose de 200 mg/kg/jour était significativement plus efficace que le placebo ($P = 0.038$). Aucun effet secondaire sérieux n'a été noté. Des effets secondaires modérés, principalement digestifs, ont été observés dans 10% (placebo et 100 mg/kg/jour), 24% (200 mg/kg/jour) et 42% (400 mg/kg/jour) des cas; seuls 5 cas ont nécessité un arrêt de la thérapeutique. Deux chiens (un à 100 mg/kg/jour et un à 200 mg/kg/jour) ont refusé de prendre le traitement. En conclusion, PYM00217 à 200 mg/kg apparaît être un traitement efficace, appétent et bien toléré pour la dermatite atopique du chien.

Resumen: En este estudio se evaluó PYM00217, una mezcla de extractos de plantas en propiedad, para el control de la dermatitis atópica canina. Ciento veinte perros se diagnosticaron con dermatitis atópica a alérgenos perennes (independientes de la estación climática) en base a la historia clínica, signos clínicos, resultados positivos a la prueba con alérgenos perennes y la eliminación de otras dermatosis. Los criterios de exclusión durante la selección incluyeron el haber sido tratados con antimicrobiales en la semana previa, con antihistamínicos en los 14 días previos, con glucocorticoides orales/tópicos o ciclosporina en los 28 días previos, y con glucocorticoides por vía parenteral, ácidos grasos esenciales o inmunoterapia en los 56 días precedentes. Agentes para el control de pulgas, champús y limpiadores de oídos fueron permitidos. Perros con una mínima extensión e índice de severidad (CADESI) de 25 fueron distribuidos de forma aleatoria para recibir PYM00217 (100, 200 o 400 mg/kg/día) o placebo durante 12 semanas. La reducción media en CADESI (población de intención a tratamiento) fue de 3.9% (placebo; $n = 29$), 4.4% (100 mg/kg/día; $n = 30$), 23.4% (200 mg/kg/día; $n = 29$) y 8.5% (400 mg/kg/día; $n = 29$). La reducción en el grupo tratado con 200 mg/kg/día fue significativa ($P < 0.01$). En los perros con un CADESI basal ≥ 50 los cambios medios fueron +10.6% (placebo; $n = 12$), +0.6% (100 mg/kg/día; $n = 14$), -29.3% (200 mg/kg/día; $n = 14$) y -3.4% (400 mg/kg/día; $n = 15$). La dosis de 200 mg/kg/día fue significativamente más eficaz que el placebo ($P = 0.038$). No se detectaron efectos adversos de gravedad. Efectos adversos menores observados en un 10% (placebo y 100 mg/kg/día), 24% (200 mg/kg/día) y 42% (400 mg/kg/día) de los casos fueron principalmente alteraciones gastrointestinales menores y sólo cinco de los casos necesitaron la interrupción del tratamiento. Dos perros (uno en el grupo de 100 y otro en el grupo de 200 mg/kg/día) rechazaron comer la alimentación medicada. En conclusión, PYM00217 a una dosis de 200 mg/kg/día parece ser un tratamiento efectivo, palatable y bien tolerado para el tratamiento de la dermatitis atópica canina.

Zusammenfassung Diese Studie evaluierte PYM00217, eine proprietäre Mischung von Pflanzenextrakten, für das Management von caniner atopischer Dermatitis (AD). Bei einhundertzwanzig Hunden wurde eine ganzjährige AD aufgrund entsprechender Anamnese, klinischen Symptomen, einem positiven Test für ganzjährige Allergene und Eliminierung von anderen Dermatosen diagnostiziert. Ausschlusskriterien waren eine Verabreichung von Antibiotika innerhalb der letzten 7 Tage, Antihistaminen innerhalb der letzten 14 Tage, oralen/topischen Glukokortikoiden oder Cyclosporin innerhalb der letzten 28 Tage und parenterale Glukokortikoide, essentielle Fettsäuren oder Immuntherapie innerhalb der letzten 56 Tage. Flohkontrolle, Shampoos und Ohrreiniger waren erlaubt. Hunde mit einem minimalen caninen atopischen Dermatitis Ausmaß und Schweregrad Index ('canine atopic dermatitis extent and severity index')(CADESI) von 25 wurden zufällig in Gruppen eingeteilt, um 12 Wochen lang PYM00217 (100, 200 oder 400 mg/kg/d) oder Placebo zu erhalten. Die durchschnittliche Reduzierung des CADESI (nach dem Prinzip der 'intention-to-treat population') waren 3.9% (Placebo; $n = 29$), 4.4% (100 mg/kg/d; $n = 30$), 23.4% (200 mg/kg/d; $n = 29$) und 8.5% (400 mg/kg/d; $n = 29$). Die Reduzierung in der 200 mg/kg/d Gruppe war signifikant ($P < 0.01$). Bei Hunden mit einer Ausgangsbasis des CADESI von ≥ 50 waren die durchschnittlichen Veränderungen +10.6% (Placebo; $n = 12$), +0.6% (100 mg/kg/d; $n = 14$), -29.3% (200 mg/kg/d; $n = 14$) und -3.4% (400 mg/kg/d; $n = 15$). Die Dosis von 200 mg/kg/d war signifikant effektiver als die Placebowirkung ($P = 0.038$). Keine ernsthaften Nebenwirkungen wurden festgestellt. Geringgradige Nebenwirkungen, die bei 10% (Placebo und 100 mg/kg/d), 24% (200 mg/kg/d) und 42% (400 mg/kg/d) der Fälle gesehen wurden, waren hauptsächlich geringgradige gastro-intestinale Störungen und nur bei fünf Fällen war es notwendig, die Therapie zu beenden. Zwei Hunde (jeweils einer in der 100 mg/kg/d Gruppe und in der 200 mg/kg/d Gruppe) verweigerten das medizinische Futter. Zusammenfassend scheint PYM00217 bei einer Dosierung von 200 mg/kg/d eine effective, wohlschmeckende und gut tolerierte Behandlung von caniner AD zu sein.