Masitinib for the treatment of canine atopic dermatitis: a pilot study

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For further information about masitinib (Masivet®) please contact AB Science Head Office at masivet@ab-science.com.

Abstract

There is an on-going need to identify medications suitable for the long-term treatment of canine atopic dermatitis (CAD). Masitinib mesilate is a potent and selective tyrosine kinase inhibitor of the c-KIT receptor. A strong relationship exists between the SCF/c-KIT pathway and pathogenesis of CAD, suggesting that masitinib may potentially fulfil the above role. This study reports on an uncontrolled pilot study of masitinib in CAD. Masitinib was administered orally to 11 dogs at a mean dose of 11.0±1.83 mg/kg/day (free base) for 28 days. Treatment response was assessed by evolution of clinical appearance according to a modified version of the Canine Atopic Dermatitis Extent and Severity Index (mCADESI), pruritus scale and surface area of lesions. Masitinib improved CAD with a mean reduction in mCADESI of 50.7±29.8% (95% C.I. = 29.4 - 72.0; p = 0.0004) at day 28 relative to baseline, with 8/10, 8/10 and 4/10 dogs showing improvement of ≥33%, ≥40% and ≥50%, respectively. Improvement was further evidenced by a decrease in pruritus score and the surface area of lesions. No serious or severe adverse events occurred during this trial, although 6/11 dogs presented with mild to moderate treatment related adverse events. There is sufficient compelling evidence to warrant further investigation.

Keywords:
Canine atopic dermatitis, tyrosine kinase inhibitor, c-KIT, masitinib, pilot study

Abbreviations:
AE = adverse event.
CAD = canine atopic dermatitis.
CsA = cyclosporin A.
D0, D12, D28 = baseline, Day-12, Day-28, respectively.
FAK = Focal Adhesion Kinase.
FcεRI = High-affinity receptor for Immunoglobulin E.
FGFR3 = Fibroblast Growth Factor Receptor 3.
GC = glucorticoid steroids.
IC_{50} = Half inhibitory concentration.
IgE = Immunoglobulin E.
Lyn = V-yes-1 Yamaguchi sarcoma viral related oncogene homolog.
mCADESI = modified Canine Atopic Dermatitis Extent and Severity Index.
PDGFR = Platelet-Derived Growth Factor Receptor
SAE = serious adverse event.

Introduction

Canine atopic dermatitis (CAD), also commonly referred to as allergic dermatitis or atopy, is a chronic skin disease that occurs in the majority of breeds. As such, it has been the focus of much research with many of its aspects periodically reviewed (Olivry *et al*., 2001a). CAD is formally defined as a genetically predisposed inflammatory and pruritic allergic skin disease with characteristic clinical features, most commonly associated with IgE antibodies to environmental allergens. Its severity can range from an annoyance in the form of mild pruritus (itching), through to debilitating extensive lesion coverage that is of great distress and can lead to self-trauma, e.g. excoriations and alopecia. Regardless of whether the condition is mild or severe, it has a negative impact on the quality of life. The prevalence of CAD is still poorly defined. Various studies have given estimates ranging from 30% to 3% (Hillier & Griffin, 2001), with a commonly cited rate being approximately 10%, whilst others have ranked it as the second most common cause of canine pruritus (Scott *et al*., 2001).

The complex aetiopathogenesis of CAD is reflected in the individually tailored, combination therapeutic treatments often required for its effective management (Nuttall, 2008). Strategies commonly employed include: allergen avoidance; allergen-specific immunotherapy (Griffin & Hillier, 2001); antihistamine pharmacotherapy (DeBoer & Griffin, 2001); antimicrobial therapy for secondary infections; essential fatty acids (Olivry *et al*., 2001b); glucorticoid pharmacotherapy (Olivry & Sousa, 2001a); and nonsteroidal anti-inflammatory pharmacotherapy including immunosuppressive drugs (Marsella & Olivry, 2001). Of these, glucorticoid steroids (GC) and cyclosporin A (CsA) were the most effective drugs available for the treatment of CAD (Olivry & Sousa, 2001b). In choosing which interventions to use, it is important to recognise that CAD is likely to be a life-long condition that typically manifests itself before 3-years of age (Griffin & DeBoer, 2001). The consensus regarding long-term therapy is that treatment should aim to keep an animal in remission and not be used intermittently to manage exacerbations (Nuttall, 2008). This criterion further complicates the process for determining an optimal treatment regimen. For example, GC is associated with numerous detrimental side effects and the risk that its benefits may be outweighed by potential complications (Nuttall, 2008; Olivry & Sousa, 2001a). This is especially true for long-term treatment regimes. CsA

is generally well tolerated, although not entirely free of side effects. Adverse events (AE) include: transient vomiting, nausea, soft stools or diarrhoea, anorexia, weight loss, cutaneous papillomatosis, hyperplastic gingivitis, periodontitis, hirsuitism, alopecia, lameness and muscle tremors, erythema and oedema of the ears (Nuttall, 2008; Diesel & Moriello, 2008; Marsella & Olivry, 2001). In addition, long term use of CsA may be associated with an increased risk of developing secondary neoplasms including skin tumours and lymphoma (Callan, 2005; Blackwood, 2004). Moreover, the relatively high cost of this medication may be prohibitive for long-term therapy. Thus, there exists an on-going need to identify alternative or complementary treatments for CAD that demonstrate high efficacy, low toxicity and are affordable.

Masitinib mesilate (Masivet®) is a protein-tyrosine kinase inhibitor (currently approved by the EMEA as a treatment of grade II/III non resectable canine mast cell tumour) that may potentially fulfil these criteria. Masitinib potently and selectively inhibits both mutated (juxtamembrane region) and wild-type forms of human and murine c-KIT (CD117) receptor *in vitro*, with an half inhibitory concentration (IC_{50}) of approximately 200 nM (Dubreuil et al., 2009). It is also shown to inhibit PDGFRα, PDGFRβ, and to a lesser extent FGFR3 and the FAK activation pathway, without inhibiting kinases of known toxicities. Another potentially important target of masitinib is Lyn (IC_{50} of 500 nM). This intracellular kinase interacts with the FcεRI and is a key component of the transduction pathway leading to IgE induced degranulation (Gilfillan & Tkaczyk, 2006). Indeed, masitinib has been shown to strongly inhibit the *in-vitro* FcεRI-mediated degranulation of human cord-blood-derived mast cells (Dubreuil *et al*., 2009).

Numerous inflammatory cells are involved in the pathogenesis of CAD, with mast cells now being considered as one of the major players (Kinet, 2007; de Mora *et al*., 2006; Hill & Olivry, 2001). Canine mast cells are known to produce a variety of inflammatory mediators that are in part responsible for the complex inflammatory cascade associated with allergic disease. Moreover, since mast cells are widely distributed throughout the body, hypersecretion of their inflammatory mediators ultimately results in many of the clinical symptoms of CAD (Hill & Olivry, 2001). Stem cell factor (SCF), the ligand of the c-KIT receptor, is a critical growth factor for mast cells, fundamental to their survival, proliferation, differentiation, adhesion and

degranulation processes (Reber *et al*., 2006). In addition, the c-KIT signalling acts synergistically with IgE receptor activation to induce mediators and cytokine release involved in CAD. Thus, there exists a strong relation between the SCF/c-KIT pathway and pathogenesis of CAD. It is hypothesised that if this link is disrupted through the inhibitory action of masitinib on c-KIT tyrosine kinase activity, then dermatological diseases such as CAD could be controlled. The purpose of this pilot study was to evaluate the potential response and safety of masitinib in the treatment of CAD.

Materials and Methods

Study design and subject recruitment

This was a prospective, uncontrolled, open label, multicentre pilot study of masitinib in dogs with atopic dermatitis, followed over a 28-day period. Dogs diagnosed with CAD in accordance to the Willemse/Prélaud criteria (Prélaud *et al*., 1998), were recruited from two veterinary dermatology clinics in Texas, U.S.A.. Dogs of any breed or sex were eligible and stayed with their owners throughout the study, under their usual housing, feeding and watering conditions. The protocol was conducted in accordance to the Good Clinical Practice Guidelines, under Investigational New Animal Drug (number INAD 11206 G-0002).

Treatment response was primarily based upon the evolution of clinical appearance. For a given dog the response parameters were recorded on the first day of treatment (D0 or baseline), prior to administration of masitinib, and then again after 14 days (D14) and 28 days (D28) of treatment by a single investigator. A full clinical examination and blood tests (glucose, urea, creatinine, SGPT, SGOT, alkaline phosphatase, complete blood count and buffy coat smear) were performed at each visit, along with measurement of body weight, body temperature and acquisition of skin photos. AEs were recorded on the first day of treatment prior to masitinib administration (D0 or baseline), and then again at D14 and D28.

Dogs were eligible to participate if an intradermal skin test (consisting of 68 allergens) performed within the last 6 months, confirmed hypersensitivity to environmental allergens. Other inclusion criteria required the dog to satisfy at least three of the following conditions: first symptoms of CAD between the ages of 6 months to 3 years; corticosteroid responsive pruritus; bilateral erythematous

interdigital pododermatitis; bilateral otitis externa; and cheilitis. Exclusion criteria applied to dogs that were: younger than 6 months; weighed less than 4.2 kg; lactating, pregnant or used for breeding; experiencing severe renal insufficiency or significant hepatic impairment or an absolute neutrophil count below 3000/mm^3; had a modified Canine Atopic Dermatitis Extent and Severity Index (mCADESI) <20, a life expectancy of less than 3 months or a medical condition that could interfere with disease evaluation. Dogs were also excluded if the following treatment washout periods had not been observed prior to entry into the study: vitamin E or fatty acids (supplement or in food) within 1 or 4 weeks respectively; anti-inflammatory or antipruritic drugs within 2 weeks; corticosteroid medication by oral or topic route within 2 weeks; long acting corticosteroid or steroid medication within 2 or 3 months respectively; short-term or long-term CsA treatment within 4 weeks or 6 months respectively; and hyposensitizing therapy within 3 months. Prior to enrolment, the investigator checked with the owners that their animal was maintained under conditions appropriate for the species and the owner signed an Owner Information/Consent form.

Treatments

Masitinib was provided by AB Science (France) in 100 mg, non-divisible capsules. Each dog was administered per os a once daily dosage of approximately 12.5 mg/kg masitinib mesilate (equivalent to approximately 10 mg/kg of the free base of masitinib). This initial dosing decision was based upon toxicity and bioavailability studies in dogs and rats (our unpublished observations). These pharmacokinetic studies established a No Observed Adverse Effect Level (NOAEL) in beagle dogs for orally administered masitinib of 15 mg/kg. At an oral dose of 10 mg/kg, the maximum concentration (Cmax) of masitinib in serum reached 794 ± 94 ng-eq/g for males and 901 ± 63 ng-eq/g for females; equivalent to 1.3 and 1.5 µM, respectively. Treatment was administered for 28 days with the dosage reviewed after 14 days of treatment. Dosage could be increased by a single capsule in the event of an insufficient response accompanied by an absence of toxicity; likewise, the dose could be reduced in the event of mild or moderate toxicity when accompanied by a positive response.

Concomitant treatments included shampooing and adulticide flea medication, applied once per week and once per month throughout the study duration, respectively. Concomitant use of insect growth regulator was also permitted. The following treatments of CAD were prohibited during the study: steroid treatments; anti-histaminic agents; CsA; tacrolimus; essential fatty acids supplementation; vitamin E supplementation; anti-pruritic agents; and immunotherapy.

Outcome measures

The degree of CAD was classified according to a modified CADESI that was based upon CADESI-02 (Olivry *et al*., 2002a; Olivry *et al*., 1997), a pruritus severity scale and skin photographs for estimation of the surface area of lesions (expressed as a percentage of body-surface). The mCADESI is a composite index that associates five clinical criteria of CAD, namely: erythema, papules, lichenification, excoriations and scraping alopecia (self inflicted alopecia). Each criterion was evaluated in 42 sites across the dog's cutaneous surface and assigned a score from 0 to 3 corresponding to absence, mild/rare, moderate/few, or severe/important presence, respectively. The total score could range from 0 to 630, with a decrease in score between two time-points indicating clinical improvement of CAD. It is noteworthy that the mCADESI used for this study reflects many of these changes implemented to a recently refined and validated version of the CADESI (CADESI-03) (Olivry *et al*., 2008; Olivry *et al*., 2007). The severity of pruritus was classified using a scale from 0 to 4 corresponding to absence, mild, moderate, severe and very severe, respectively. A decrease in score between time-points is representative of improvement. Scores were based upon the owner's perception, the frequency and the persistence of scraping, nibbling, rubbing or licking movements. Evaluation of treatment response was made by comparing the initial mCADESI and pruritus assessments at baseline (D0) with those made at subsequent examinations, e.g. at D28.

The primary response outcome was the reduction in mCADESI score after 28 days (D28) of treatment, expressed as the mean difference in mCADESI at D28 with respect to the baseline (D0), and as the proportion of dogs achieving improvement in severity of CAD in this time. *A priori* thresholds of mCADESI improvement were defined at 33%, 40% and 50%. Secondary outcomes included the proportion of dogs achieving improvement in severity of CAD from between D0 and D14, as measured

by the mCADESI score, and the proportion of dogs achieving improvement in severity of pruritus from between D0 to D28, as assessed by the pruritus score. *A priori* thresholds of pruritus improvement were defined as: (i) a decrease in score of at least 1 and a final score ≤2; (ii) a decrease ≥50% of the initial pruritus score; and (iii) a final pruritus score ≤2. The proportion of dogs achieving improvement in surface area of lesions from between D0 to D28 was also analysed, as was a breakdown of the improvement in mCADESI subscores at D14 and D28 with respect to baseline.

Safety and tolerability assessment

Safety was assessed by occurrence of AE, and monitoring haematological and biochemical parameters during the study period. A full clinical examination and blood tests were performed at each visit. Toxicity was graded according to a modified version of the Eastern Cooperative Oncology Group criteria (Oken *et al*., 1982), using a scale from 0 to 4 ranging in severity from no effect to very severe toxicity. The relationship of each AE to the study drug was assessed by the treating veterinarian. The minimal haematological and biochemical requirements to continue dosing were as follows: absolute neutrophil count >1000/mm^3; haematocrit >20%, platelets >100,000 /mm^3; liver transaminases ≤5 × upper limit of normal; and serum creatinine <3.5 mg/dL.

Statistical analysis

The variations of total and subtotal scores of mCADESI between D0 and D14 or D28 were compared using the Student's t-test. Wilcoxon test for paired variables was used for confirmation. Differences were considered significant at $p < 0.05$. Statistical analysis was performed using SAS software 8.2 (Chicago, IL).

Results

Baseline characteristics and participant flow

Between 05 August and 14 December 2004, a total of 13 dogs from two different centres were screened for participation. Eleven of these were subsequently enrolled, two dogs being omitted due to either insufficient body weight or a negative intradermal skin test. Participant baseline characteristics are presented in Table 1.

Table 1. Baseline characteristics.

Characteristic	Subject											Summary	
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11		
Weight (kg)	9.5	35.2	22.9	15.0	37.5	13.1	41.4	41.4	24.3	18.2	31.8	Mean ± SD min - max	26.4 ± 11.6 9.5 – 41.4
Age (y)	3.4	2.2	3.3	7.9	3.0	0.7	3.9	4.7	0.9	4.7	4.9	Mean ± SD min - max	3.6 ± 2.0 0.7 – 7.9
Gender	F	M	M	M	F	M	M	M	F	F	F	Male Female	6/11 (55 %) 5/11 (45 %)
Neutered	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Yes No	10/11 (91 %) 1/11 (9 %)
Pre-treated with antibiotics*	Y	Y	N	N	Y	Y	Y	Y	N	N	N	Yes No	10/11 (91 %) 1/11 (9 %)
Possible secondary skin infection	Y	Y	N/A	N/A	Y	N/A	N/A	N/A	Y	Y	N	Yes No or N/A	5/11 (45 %) 6/11 (55 %)
mCADESI (D0)	80	101	74	112	189	99	143	52	29	55	56	Mean ± SD min - max	90.0 ± 46.0 29 - 189
Pruritus (D0)	2	2	2	3	4	3	4	3	2	2	4	2 score 3 score 4 score	5/11 (46 %) 3/11 (27 %) 3/11 (27 %)
Surface area of Lesions (D0)	50-75%	50-75%	50-75%	>75%	50-75%	>75%	25-50%	<25%	<25%	50-75%		<25% 25-50% 50-75% >75%	2/11 (18 %) 1/11 (9 %) 5/11 (45 %) 2/11 (18 %)

N/A: not assessable

* Antibiotic treatment was stopped at inclusion and no dog was treated by antibiotic during the study.

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Table 2. Efficacy parameters and dosing levels: according to subject

		S1	S2	S3	S5	S6	S7	S8	S9	S10	S11
Dose (mg/kg/day)[a]		=	+	-	+	+	=	=	-	=	=
	D0-D13	10.5	11.3	13.1	10.6	7.6	10	12.1	12.3	10	10
	D14-D28	11	14	8.8	13.5	14.5	10	12.3	8.3	10	10
mCADESI score (Improvement,%)[b]	D14	56 (30%)	78 (23%)	50 (32%)	125 (34%)	105 (-6%)	101 (29%)	20 (62%)	8 (72%)	4 (93%)	48 (14%)
	D28	32 (60%)	86 (15%)	43 (42%)	102 (46%)	58 (41%)	77 (46%)	5 (90%)	29 (0%)	4 (93%)	15 (73%)
Pruritus score	D0	2	2	2	4	3	4	3	2	2	4
	D28	1	2	1	2	3	2	1	3	3	4
	Improvement	50%	0%	50%	50%	0%	50%	66%	-50%	-50%	0%
Surface area of Lesion[c]	D0	50-75%	50-75%	50-75%	>75%	50-75%	>75%	25-50%	<25%	<25%	50-75%
	D28	<25%	<25%	<25%	50-75%	<25%	25-50%	<25%	<25%	<25%	50-75%

S4 not included in response analysis due to early withdrawal from study.

[a] Dose according to free base of masitinib; no dose adjustment (=); dosage increase (+); dosage decrease (-). Increment of ± 1 capsule (100 mg). Note: the variation in dose for those subjects maintained under a constant dosage is due to weight change.

[b] mCADESI improvement relative to baseline.

[c] Surface area of lesion according to percentage of body surface.

Table 3. Efficacy parameters and dosing levels: summary statistics (n=10)

Dose (mg/kg/day)[a]		Mean ± SD	11.0 ± 1.86		
		Range	7.6 – 14.5		
mCADESI improvement (%)[b]	D14	Mean ± SD	38.5 ± 29.2		
		95 % C.I	17.5 – 59.1		
		p-value	0.0025		
	D28	Mean ± SD	50.7 ± 29.8		
		95 % C.I	29.4 – 72.0		
		p-value	0.0004		
Pruritus score (n, %)		Score 1	Score 2	Score 3	Score 4
	D0	0/10 (0 %)	5/10 50(%)	2/10 (20%)	3/10 (30 %)
	D28	3/10 (30 %)	3/10 (30 %)	3/10 (30 %)	1/10 (10 %)
Surface area of Lesion[c] (n, %)		<25%	25-50%	50-75%	>75%
	D0	2/10 (20 %)	1/10 (10 %)	5/10 (50 %)	2/10 (20 %)
	D14	5/10 (50 %)	3/10 (30 %)	2/10 (20 %)	0/10 (0 %)
	D28	7/10 (70 %)	1/10 (10 %)	2/10 (20 %)	0/10 (0 %)

See Table 2 for explanation of footnotes.

The breeds of dog in the study included: Labrador Retriever (3); Mixed (3); and one each of Cocker Spaniel, Ori Pai, Beagle, Standard Poodle and Gordon Setter. Safety analysis was performed on all 11 dogs enrolled; however, one dog (S4) was withdrawn a week after inclusion due to an AE and consequently only the ten dogs that completed the study were eligible for response analysis. Protocol deviations were related to dosage modification or absence of dosage modifications primarily concerning the protocol guidance to increase dosage in case of insufficient response in the absence of toxicity. As a consequence, some subjects may not have benefited from an optimal dosage.

Treatment response

Masitinib was administered per os at a mean dosage of 11.0 ± 1.83 mg/kg/day (expressed as free base of masitinib unless otherwise stated), with median treatment duration of 28 days (27 - 31 days). Evaluation of the response parameters mCADESI, pruritus score and surface area of lesions, along with the dosing history are presented in Table 2 and 3.

Treatment with masitinib significantly improved the severity of CAD with a mean reduction in mCADESI of 50.7 ± 29.8% (95% C.I. = 29.4 - 72.0; p = 0.0004) at day 28 relative to baseline. A significant improvement was also observed at D14 relative to baseline, (38.3 ± 29.2%; 95% C.I. = 17.5 - 59.1; p = 0.0025). These results were confirmed by a non-parametric test (Wilcoxon signed rank test). All subjects demonstrated a reduction in their mCADESI scores between baseline and D28, with one exception. Subject S9 initially showed improvement at D14 (-72%), but by D28 their mCADESI score had returned to its baseline value. This relapse was most probably due to a decrease of masitinib dosage at D14, from 12.3 to 8.3 mg/kg, in response to a moderate AE (neutropenia). Another subject, S6, initially showed no response in their mCADESI score at D14 at masitinib dose of 7.6 mg/kg. Increasing this dosage to 14.5 mg/kg (one additional capsule) resulted in a 41% reduction of mCADESI by D28. The *a priori* scores for mCADESI of ≥33, ≥40 or ≥50% improvement were observed for 8/10 (80%), 8/10 (80%) and 4/10 (40%) subjects, respectively. Significant improvement in the subscores of mCADESI at D28 where obtained for erythema (p = 0.0005), lichenification (p = 0.0371), excoriation (p = 0.0177) and scraping alopecia (p = 0.0137). Indeed, significant improvement in

excoriation (p = 0.0138) and scraping alopecia (p = 0.0102) were already evident at D14. This analysis also revealed was that erythema, lichenification and excoriations were the highest contributing subscores for the decrease of total mCADESI score at D28 (data not shown).

Pruritus was evaluated at baseline and D28 (Table 2). Pruritus showed an overall improvement, the proportion of subjects with a pruritus score of 1 increasing from 0/10 (0%) to 3/10 (30%), respectively, whereas the proportion of subjects with a pruritus score of 4 decreased from 3/10 (30%) to 1/10 (10%), respectively. The *a priori* scores for pruritus were as follows:

- 5/10 subjects (50%) experienced a ≥1 decrease of pruritus score and a final score ≤2;
- 5/10 subjects (50%) had their pruritus score decrease ≥50%; and
- 6/10 subjects (60%) had a final score ≤2.

The surface area of lesions decreased between inclusion and D28; <25% of body surface for 7/10 subjects (70%) at D28, in comparison with only 2/10 subjects (20%) at inclusion. At D28, no patient had a surface area of lesions >75%, compared to 2/10 subjects (20%) at D0.

Safety and tolerability

Assessment of masitinib safety was performed on all 11 dogs enrolled into the study. The duration of treatment was between 5 – 31 days, with median treatment duration of 28 days. Seven AEs assessed as being at least possibly related to masitinib, were observed for 6/11 dogs (55%), all of which were classified as mild or moderate in severity, with no severe or SAE reported (Table 4). Only one AE led to treatment arrest, a febrile episode lasting 2 days in subject S4, which cured without sequelae. This 8-year-old dog presented with fever at 104.5°F (40.3°C) associated with lethargy 5 days after commencement of masitinib treatment at 12.9 mg/kg/day. Fever was associated with a high absolute neutrophil count (10540/mm^3, normal ranging from 3000 to 11500). Masitinib was discontinued on the fifth day of treatment, and fever resolved 2 days later. Although the chronological data seemingly implicates masitinib to this AE, a direct relationship seems unlikely since masitinib is known to induce neutropenia rather than high neutrophil count; thus, a non-drug related infection could not be ruled out.

Table 4. Adverse events at least possibly related to treatment.

Subject	AE description	* Onset (days)	Intensity	Outcome
S1	Urinary incontinence	12	Moderate	Unchanged
S4	Fever 104.8°F, lethargy	5	Moderate	Recovered
S7	Lameness of left leg/hip	7	Mild	Recovered
S8	Upset stomach, loose stool	17	Moderate	Recovered
S9	Soft stool	1	Mild	Unchanged
	Neutropenia	14	Moderate	Improving
S11	Neutropenia	15	Moderate	Resolved

* Delay from first intake of masitinib (days).

Table 5. Biological exams: haematology

Haematological parameters	D0	D14	D28
White blood cells/µL, n	11	10	10
Mean ± SD	9110 ± 2570	6330 ± 2380	7280 ± 3290
Red blood cells x 10^6/µL, n	11	10	10
Mean ± SD	6.61 ± 0.63	6.40 ± 0.58	5.94 ± 0.68
Neutrophils/µL, n	11	10	10
Mean ± SD	6538 ± 2360	4063 ± 2042	5571 ± 3058
Lymphocytes/µL, n	10	10	10
Mean ± SD	1344 ± 404	1335 ± 484	1112 ± 482
Monocytes/µL, n	10	10	10
Mean ± SD	534 ± 313	551 ± 286	371 ± 213
Eosinophils/µL, n	9	10	9
Mean ± SD	311 ± 152	317 ± 195	243 ± 120
Basophils/µL, n	4	3	5
Mean ± SD	48 ± 56	63 ± 55	0 ± 0
Platelets x 10^3/µL, n	8	9	9
Mean ± SD	287 ± 123	231 ± 105	214 ± 110

A summary of haematological parameters is presented in Table 5. As expected, a slight decrease of cells of the white lineage related to the effect of masitinib was observed. An average decrease of 36% in neutrophils was noted at D14, however, at no time during the study did any subject have a neutrophil level below 1000/μL, and at D28 the decrease was partly improved (average decrease of 12%). Platelets decreased by 10% and 22% at D14 and D28, respectively. Lymphocytes, monocytes, eosinophils, and basophils are immune and inflammatory cells associated with the pathophysiology of CAD, all of which registered some decrease at D28.

Discussion

To compare the primary outcome of this study (i.e. an improvement in mCADESI of 50.7% [95% C.I. = 29.4 - 72.0] after 28 days of treatment relative to baseline) with related clinical trials in CAD, it is necessary to take into consideration differences between evaluation mechanisms. The mCADSEI score employed for this study incorporates similar information as its parent version, albeit with additional evaluated lesions (5 vs. 3) and body sites (42 vs. 40). Importantly, three of the most contributing subscores for the primary outcome are common to the standard and mCADSEI versions. It is therefore relatively straightforward to reconstitute the equivalent scores from the standard CADESI scale and thereby, make legitimate comparisons with studies using this scale. Thus, re-evaluation using the standard CADESI score yields a 54% improvement in the primary outcome. In a randomised controlled trial that investigated the efficacy of CsA in CAD (Olivry *et al*., 2002b), the mean reduction of CADESI after 6 weeks was 34% in the placebo group, 41% in the low-dosage (2.5 mg/kg) CsA group and 67% in the high-dosage (5 mg/kg) CsA group. In another study comparing CsA (5 mg/kg) against prednisolone (0.5 mg/kg) (an example of a synthetic glucocorticoid), the mean reduction of CADESI after 6 weeks was 58% in the CsA group and 69% in the prednisolone group (Olivry *et al*., 2002a). Similarly, a study comparing CsA (5 mg/kg) against methylprednisolone (0.75 mg/kg) reported a decrease in CADESI score after 16 weeks equal to 52% in the CsA group and 45% in the methylprednisolone group (Steffan *et al*., 2003). Bearing in mind that these trials are not strictly comparable to the present study, e.g. differences between their

methodology and exposure times, the comparison suggests that masitinib might have an efficacy on CAD, comparable to methylprednisolone or high-dosage CsA.

Masitinib was relatively well tolerated with seven mild to moderate drug-related AEs and no severe or SAE reported. The majority of haematological and biochemical parameters were not significantly modified during the study. The partial recovery in neutrophil level at D28 probably indicates secondary bone marrow stimulation and growth of neutrophil progenitors less sensitive to c-KIT inhibition. The decrease in various haematological parameters could explain, at least in part, the effect of masitinib on CAD. Alternatively, they may reflect the efficacy of masitinib on the disease and a secondary reduction of these inflammatory cells. Of particular note was the effect of masitinib on basophils at D28, which disappeared from blood circulation in all those patients with available data (5/5). This suggests that follow-up of basophil numbers could serve as an index for efficacy of masitinib in CAD.

Although this study was not intended to investigate the optimal therapeutic dose of masitinib in CAD, there was evidence to suggest a dose-dependent response and that dosages ≥10 mg/kg are necessary for a discernable improvement of CAD or pruritus. This threshold of activation might be related to pharmacokinetic parameters, i.e. the in situ concentration of free masitinib should be above the IC_{50} of c-KIT, and Lyn inhibition. The implication here is that an increased dose, within the limits of acceptable tolerability, would have further improved response.

Considering the combined outcomes of mCADESI and pruritus, then 5/10 subjects, experienced both a decrease of ≥1 in pruritus score with a final score of ≤2, and an improvement of ≥33% in mCADESI. Conversely, in 4/10 subjects the mCADESI score decreased while pruritus remained stable or increased. Of these, 3/4 subjects had either been receiving antibiotic treatment at inclusion to the study and/or had visual evidence of cutaneous secondary infection (see Table 1). It was further observed that successful treatment of pruritus occurred more frequently in those dogs that had received antibiotic treatment before inclusion in the study. In contrast, no difference according to previous antibiotic treatment was observed for improvement of mCADESI score. This suggests that unresolved skin infections were a complicating factor in this study, exacerbating the subject's allergic status and most likely leading to a reduced treatment response in pruritus. Hence, to avoid confounding effects from cutaneous infections, future studies should either adopt

suitable exclusion criteria or concomitant antibiotic treatment (e.g. cephalexin, amoxicillin-clavulanate) of bacterial infection.

Other conditions producing similar symptoms to CAD are flea, food or contact allergy dermatitis. Possible confounding results associated with these were avoided or mitigated respectively, by the administration of flea treatment (adulticide and insect growth regulator), by the exclusion of subjects having a negative intradermal skin test, or via a weekly shampooing regimen. The absence of a definite food allergy status in the present study leaves the possibility of concomitant CAD and food allergy, which may result in a reduced treatment response. Shampoos are widely considered as the minimal standard symptomatic treatment in the management of mild dermatological conditions, providing immediate relief of pruritus by eliminating contact allergens. However, most shampoos have little residual activity and are generally regarded as adjunctive treatments, rarely effective as the sole therapy (Randall, 2005). Moreover, given the shampooing regimen followed, any shampoo-related benefits to CAD would have been present at baseline, further reducing its short-term influence.

Within the limitations of such an uncontrolled open pilot study, these response data give a promising indication of masitinib's potential for the treatment of CAD. The frequency of possible treatment related AEs was relatively high at 55%, however, all were mild or moderate in severity and led to only one treatment discontinuation. Given masitinib's selective mechanism of action, the results of this study also help to further establish the critical role of mast cells in the pathogenesis of CAD. More specifically, it supports the viability of exploiting the SCF/c-KIT and Lyn pathways as a therapeutic target. In conclusion, there is sufficient compelling evidence to proceed with a more formal placebo controlled clinical trial. The design of such a study would ideally incorporate stricter measures to avoid possible confounding effects, for example, exclusion of dogs with: exclusive seasonal allergies; food allergies; or clinical signs of infection for Staphylococcus, Malassezia, or any ectoparasite infection.

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