

Pharmacokinetics of masitinib in cats

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Abstract Masitinib is the first veterinary drug recently approved in Europe to treat mast cell tumours in dogs (Hahn et al. JVIM, Masivet). This inhibitor is selective and highly efficient in blocking c-Kit, PDGFR, and Lyn tyrosine kinase activities. It showed good efficacy and acceptable toxicity in several animal studies such as mice, rats, rabbits and dogs (Dubreuil P, et al. submitted, and Hahn et al. (J Vet Intern Med 22(6):8, 2008)). C-kit is a tyrosine kinase receptor that plays a critical role in the biology of mast cells including differentiation, survival, migration and cytokine/mediator release. Mast cells are involved in a number of allergy-and immune-related diseases in cats such as asthma (Reinero Carol et al. Vet Immunol Immunopathol 121(3–4):9, 2008), inflammatory bowel disease, (Janeczko et al. Vet Mic 128(1–2):15, 2008), and feline mast cell tumours (Rassnick et al. J Am Vet Med Assoc 232(8):1200–1205, 2008). Therefore, there might be a strong rationale to use masitinib in these indications. Here, we report the results of a preliminary pharmacokinetic study of masitinib in cats which showed a good bioavailability of ~60% in both sexes. We propose that an oral dose of 10–15 mg/kg masitinib is appropriate to achieve adequate plasma concentrations.

Keywords Pharmacokinetics · Cat mast cells · Tumours · Masitinib · Asthma · Inflammatory bowel disease

Introduction

The understanding of cellular dysfunction at the biochemical level has promoted the emergence of targeted therapeutic approaches in cancer, allergy and inflammatory disorders

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in human medicine, and more recently in veterinarian medicine. As a first example of targeted therapy in dogs, masitinib has been recently approved by the EMEA (EPAR 2008) to treat mast cell tumours (MCT), the result of uncontrolled mast cell proliferation. Masitinib is an ATP competitor that selectively targets c-Kit and PDGF tyrosine kinase receptors with high affinity (150 nM (Hahn et al. 2008) and 450 nM, respectively), and Lyn tyrosine kinase, and potentially inhibits their enzymatic activity. The receptor of stem cell factor, c-Kit, is expressed on mast cells and is critical for their differentiation, survival, migration and mediator/cytokine release (Lennartsson et al. 2005). In a phase 3 study in dogs with non-resectable or recurrent grade II or III tumours, masitinib (12.5 mg/kg/day) significantly prolonged time-to-tumour progression (TTP) compared to placebo (75 versus 118 days, $P=0.038$). In non-resectable tumours, the TTP increased from 75 to 173 days, ($P=0.001$). A significant increase in the overall survival (OS) rate in non-resectable tumours was achieved with masitinib after one (62.1% versus 36.0%, $P=0.024$), and two years (39.8% versus 15.0%, $P=0.121$) as compared to placebo. These results were obtained with little associated toxicity showing that masitinib is safe and effective at delaying tumour progression in dogs.

Mast cells (or mastocytes) are resident cells in several types of tissues. Their activation via the IgE receptor, in synergy with c-Kit activation, triggers inflammatory responses, release of vasoactive substances such as histamine and heparin, chemokines and cytokines that attract and activate other immune cells (Kirschvink et al. 2007). Therefore, *in vitro* treatment of mast cells by masitinib induced a reduction of cytokines and mediators released upon IgE receptor activation (unpublished data).

In a proof of concept study in ovalbumine-sensitised mice, masitinib (20 mg/kg, twice daily) reduced significantly the number of recruited eosinophiles in the bronchoalveolar lavage fluid, and a dose-dependent reduction of pulmonary mast cells in lung tissue of up to 80% at 80 mg/kg was observed (unpublished data). Furthermore, in a mouse model of inflammatory bowel disease (IBD), induced by intrarectal trinitrobenzene sulfonic acid (TNBS), masitinib reduced severity of the disease, histological lesions and cytokines release in a dose-dependant manner (unpublished data).

Finally, in humans, masitinib (9 mg/kg/day) has been used successfully in a clinical phase 2 study of severe corticosteroid-dependant asthma. (Humbert M, et al. 2009, Allergy, in press).

These findings provide a strong rationale to use masitinib in cat mast cell tumours, asthma, and IBD. Therefore, we performed a preliminary PK study in cats, before launching the full battery of safety and pharmacodynamic studies, and we compared the results to those obtained in rats and dogs.

Materials and methods

Animals Three male and three female cats (age 35.5 ± 1.6 months, 3.9 ± 0.9 kg) were from Harlan Sprague Dawley Inc. (Indianapolis, USA) and Isoquimen (Sant Feliu de Codines, Spain). Animal housing and care complied with the recommendations of Directive 86/609/EEC. Agreement number C 31 188 01 obtained from the French Veterinary Authorities.

Drug administration Masitinib at 5 mg/ml was dissolved in 0.9% NaCl for both i.v. and oral administrations. The first administration of masitinib was delivered as bolus i.v. into the cephalic vein using a plastic syringe fitted with an infusion set. The second administration was by gavage using an oesophageal cannula and a plastic syringe under short isofluran anaesthesia. Each animal was observed daily and abnormal findings were recorded as they were observed.

Analyses After i.v. and oral administrations, blood samples were collected (pre-dose, 0.25, 0.5, 1, 2, 4, 8, 12, 24 and 48 hours after treatment). Lithium-heparinised cat blood was centrifuged and plasma precipitated with methanol. The supernatant was diluted with acetonitrile and dried under nitrogen. The residues were dissolved in water and analysed by LC/MS/MS (API 4000, Applied Biosystem, France) with TEA 10 mM at pH 10.5 as mobile phase. Lithium-heparinised cat plasma (Valley Biomedical, USA), spiked with masitinib, served as calibrator. The analytical method used in this non-GLP study is derived from a fully validated method in rats and dogs and has been partially validated in cats. It allows for the measurements of concentrations of masitinib from 1 to 500 ng/ml in lithium heparinised cat plasma.

Results

The NOAEL (No Observed Adverse Event Level) has been established at 8.4 mg/kg/day during long term toxicity studies in both rats (26 weeks) and dogs (39 weeks) (Internal reports No; 26099TCR, and No. 26100TCS, respectively, and the EMEA (EPAR 2008)) and the approved dose in dog is 12.5 mg/kg. Consequently, we selected the dose of 10 mg/kg for this PK study (Table 1).

Three male and three female cats received a single i.v. administration of 10 mg/kg masitinib. After a wash-out period of seven days they received a second administration of the same dose by gavage. The main results are given in Table 2.

The bioavailability of masitinib was found to be 56% in females and 61% in males. No gender difference in the AUCs was observed in cats and the exposure was comparable. The measured half life between 3–5 hours after administration could be considered compatible with single daily dosing.

Depending on the route of administration, initial differences in the plasma concentrations were observed (Fig. 1) but four hours after medication, similar concentrations were reached. After i.v. administration of masitinib, transient clinical signs (mydriase, hypotension) were observed in all animals, and loss of consciousness in some animals occurred shortly after injection with the disappearance of all clinical signs one hour later for the majority of animals. In contrast, after oral dosing, masitinib was well-tolerated in male and female cats without overt side effects. Figure 1 shows the plasma concentrations ($C_{\max}$) of masitinib depending on the route of administration. A peak was reached orally after two hours in cats as previously observed in dogs (Table 3). Although the concentration was lower in cats, 465 ng/ml ($\sim 1 \mu\text{M}$) versus 548 ng/ml in dogs, the concentration was above the IC_{50} of c-Kit (Hahn et al. 2008).

Table 1 Comparison, between rats and dogs, of AUC (ng/ml·h) in male and female animals

Rat (Day 1)*		
	males (n=12)	females (n=12)
8.4 mg/kg (NOAEL)	4110	8350
Dog (Day 1)**		
	males (n=4)	females (n=4)
8.4 mg/kg (NOAEL)	4625 $\pm$ 1175	4045 $\pm$ 1250
12.5 mg/kg (approved dose)	6500	

* CIT-study #26099, 26 week study, **CIT-study #26100, 39 week study

Table 2 Pharmacokinetic parameters in male and female cats

Administration	Cats		C _{max} (ng/ml)	T _{max} (h)	AUC ₀₋₂₄ (ng/ml•h)	t _{1/2} (h)	Clearance (liter/h/kg)	V _d (liter/kg)
i.v. 10 mg/kg	females (n=3)	mean	1884	0.33	6427	4.67	1.53	10.17
		SD	124.5	±0.14	±576.5	±1.2	±0.15	±2.06
		% CV	6.58	43.3	8.97	25.66	9.73	20.28
	males (n=3)	mean	1886	0.33	5432	4.06	1.84	10.51
		SD	8.72	±0.14	±580.0	±1.39	±0.2	±2.37
		% CV	2.58	43.3	10.68	34.18	11.13	22.58
oral 10 mg/kg	females (n=3)	mean	465	2.33	3380	505	2.88	20.67
		SD	85.3	±1.53	±146.18	±1.77	±0.17	±6.21
		% CV	18.35	65.47	4.32	35.14	6.05	30.07
	males (n=3)	mean	465	2	3191	3.02	3.2	14.06
		SD	±16.13	±0.0	±609	±0.22	±0.66	±3.8
		% CV	3.47	0	19.08	7.34	20.61	27.07

Discussion

Masitinib is the first veterinary drug (Masivet) to target specifically stem cell factor receptor c-Kit on mast cells. These tissue-resident cells play a central role in allergy, anaphylaxis and inflammatory disorders due to their secretion of cytokines and other mediators (Kirschvink et al. 2007). The primary indication of masitinib in veterinarian medicine is canine mastocytoma, the result of uncontrolled accumulation and proliferation of mast cells. These MCTs are at least in part driven by c-Kit signalling particularly in tumours exhibiting activating c-Kit mutations. The pharmacokinetic properties of masitinib offer an unprecedented opportunity to treat these tumours in dogs orally instead of surgically, especially when resection is not an option.

Mast cell tumours represent the second most frequent cancer in cats (~20%), with two distinct forms, the more frequent mastocytic form and the rarer histiocytic form, prevalent in different age groups of 10 and 2.4 years, respectively. The third common cancer is intestinal mastocytoma which affects older cats (mean age 13 years). A genetic predisposition has been proposed for the development of these tumours (Max's_House 2008). As for the canine counterpart, activated mast cells can be detected histochemically and activating mutations of their c-Kit receptor have been identified (Isotani et al. 2006).

Mast cells rarely circulate in blood but reside in most tissues of the body. Their granules contain several biologically active molecules such as histamine, serotonin, and heparin, but also inflammatory mediators (prostaglandins and leukotrienes), enzymes (metalloproteases), chemokines and cytokines, which attract and/or activate eosinophiles and other immune cells.

Fig. 1 Masitinib [10 mg/kg] plasma concentrations in cats. A comparison of two routes of administration shows an initial difference in the plasma concentrations disappears after four hours and similar concentrations were obtained

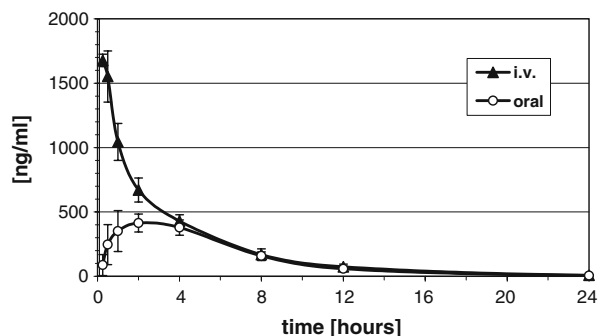


Table 3 Comparison of the main pharmacokinetic parameters in rats and dogs

Administration	Rats		C _{max} (ng/ml)	T _{max} (h)	AUC ₀₋₂₄ (ng/ml·h)	t _{1/2} (h)	Clearance (liter/h/kg)	V _d (liter/kg)
oral 8.4 mg/kg	females (n=20)	mean	675	4	8346	4.48	NA	NA
	males (n=20)	mean	385	2	4110	4.69	NA	NA

Administration	Dogs		C _{max} (ng/ml)	T _{max} (h)	AUC ₀₋₂₄ (ng/ml·h)	t _{1/2} (h)	Clearance (liter/h/kg)	V _d (liter/kg)
oral 8.4 mg/kg	females (n=4)	mean	431	2	4045	10.2	NA	NA
	males (n=4)	mean	422	2	4625	10.6	NA	NA

NA= not available

All these mediators orchestrate host defence mechanisms from inflammation to hypersensitivity reactions (Dahm 2001), which may include allergic dermatitis and asthma in cats (Roosje et al. 2004).

With an incidence in cats of about 1%, feline asthma has been diagnosed world-wide and in all ages of cats. Sometimes referred to as feline bronchial disease (FBD) because of an unclear analogy to the human disease and unknown pathophysiology, feline asthma remains a chronic progressive disease (Foster et al. 2004). Its origins are ill-defined and can be caused by infectious agents (parasites, microorganisms, viruses) or environmental hazards (Corcoran et al. 1995). Furthermore the diagnosis is not straightforward as symptoms from a parasitic infection with heartworms or bacteria such as mycoplasma resemble each other and require further tests like bronchoalveolar lavages and mycoplasma cultures. Symptomatic treatment includes anti-inflammatory drugs for the chronic inflammation of the airways, and during spells of severe coughing bronchodilators are used (Little 2001). In models of experimental feline asthma a distinction exists between eosinophilic inflammation and airway remodelling, which is a permanent architectural change of the airways (Reinero et al. 2008). Remodelling has been shown to be a consequence of the activity of mast cell-derived matrix metalloproteinases-9 in mice (Iba et al. 2007) or man (Ohbayashi and Shimokata 2005) during inflammation.

Inflammatory bowel disease (IBD) is a common condition in cats and the result of uncontrolled intestinal inflammation of poorly understood origin, which could be environmental, genetic susceptibility or via pathogens. The result is a markedly different composition of the mucosal flora in affected animals as compared to healthy ones (Janeczko et al. 2008). This population shift has important implications as it leads to the loss of bacteria-derived factors necessary for optimal mucosal health and open entry points for other opportunistic infections. Interestingly, this inflammation is not accompanied by infiltrating T-cells (Waly et al. 2004) suggesting that resident cells such as mast cells are the source of cytokines and mediators involved in IBD pathophysiology.

Consequently, targeting mast cells with masitinib should result in substantial health benefits in cats in which mast cells may play a critical role. Therefore, pharmacokinetic and toxicity studies are important endpoints to investigate in cats. It is worth noting that the established NOAEL of 8.4 mg/kg is the same in two very different species such as rats and dogs. Moreover, it has been shown that the associated toxicity is transient and generally mild in nature when administered orally. In cats, masitinib achieved similar concentrations in plasma, as compared to dogs, and reached a five-fold higher level than pharmacologically necessary to inhibit c-Kit tyrosine kinase activity (c-Kit IC₅₀=150 nM). The only side effect observed, which was found to be rapidly reversible, was linked to neurological disorders. This phenomenon was probably related to the high C_{max} resulting from i.v. administration. However, the mechanism of this neurological toxicity remains to be determined.

In dogs, the approved dose of 12.5 mg/kg masitinib leads to a calculated AUC of about 6500 ng/ml·h (Table 1). Thus, although the bioavailability is lower in cats as compared to dogs (~80%) or rats (~70%), we target a similar dose of 10–15 mg/kg masitinib which allows reaching a plasma concentration above the IC₅₀ of c-Kit (Hahn et al. 2008). The half life of masitinib was measured to be 3–5 hours, whatever the route of administration, comparable to observations in rats (4–6 hours) but shorter as compared to dogs (10–20 hours)(Hahn et al. 2008).

A comparison of the main pharmacokinetic parameters obtained in the present study with those obtained in rats and dogs with comparable oral dosing shows that no significant interspecies differences exist, apart from a longer half life in dogs. The C_{max} was between 555 and 540 ng/ml in males and females, respectively and the corresponding T_{max} of 2 hours, resulting in an AUC_{0–24} between 4625 and 4045 ng/ml·h for male and female dogs, respectively (Table 3).

In order to avoid CNS toxicity observed during i.v. administration in cats, oral treatment should be considered for further exploration of safety and efficacy of masitinib in cats.

Potential conflict of interest This study was outsourced by AB Science to: ADME Bioanalyses, 75, Chemin de Sommières – 30310 VERGEZE, France. FB, TB, and AM are employed by AB Science.

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