

Pharmacokinetics and pharmacodynamics of ramipril and ramiprilat in healthy cats

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The pharmacokinetics of ramipril and its active metabolite, ramiprilat, was determined in cats following single and repeated oral doses of ramipril (Vasotop® tablets) (once daily for 9 days) at dose rates of 0.125, 0.25, 0.5 and 1.0 mg/kg. The pharmacodynamic effects were assessed by measuring plasma angiotensin-converting enzyme (ACE) activity. Maximum ramipril concentrations were attained within 30 min following a single dose and declined rapidly (concentrations were below the limit of quantification 4 h after treatment). Peak ramiprilat concentrations were detected at approximately 1.5 h. The apparent terminal half-life ($t_{1/2\beta}$) was ≥ 20 h irrespective of the dose. Ramiprilat accumulated in plasma (ratio of accumulation 1.3 to 1.9 depending on the dose rate) following repeated administration. Steady-state conditions were attained after the second dose. Excretion was predominant in faeces (87%) and to a lesser extent in urine (11%). The rate and extent of absorption of ramipril as well as its conversion to ramiprilat were not significantly influenced by the presence of food in the gastrointestinal tract. Plasma-ACE activity was almost completely abolished 0.5–2.0 h after treatment, irrespective of the dose rate. Significant inhibition of ACE activity of 54.7 to 82.6% (depending on the dosage) was still present 24 h after treatment. Treatment was well-tolerated in all cats. Ramipril at a dose rate of 0.125 mg/kg once daily produces significant and long-lasting inhibition of ACE activity in healthy cats. The appropriateness of this dosage regime needs to be confirmed in diseased cats.

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INTRODUCTION

Ramipril is a pro-drug of the angiotensin-converting enzyme (ACE) inhibitor ramiprilat. Following oral administration to the humans, dogs and rodents, ramipril is absorbed rapidly from the gastrointestinal tract and is hydrolyzed in the liver to its active metabolite ramiprilat, which is a highly potent inhibitor of ACE (Becker & Schölkens, 1987; Bünning, 1987). In USA and Europe, ramipril has been used since the 1980s for the management of arterial hypertension, cardiac insufficiency and renal failure in humans (Becker & Schölkens, 1987; Meisel *et al.*, 1994) and for the treatment of congestive heart failure in dogs (Schölkens *et al.*, 1986; Sent *et al.*, 2000).

Angiotensin-converting enzyme inhibitors may be useful for the management of some feline ACE-dependent conditions such as chronic kidney disease and hypertension. Chronic kidney disease, also known as chronic renal insufficiency or chronic renal failure, is defined as a primary loss of excretory function prevailing for a lengthy period (months to years). This condition is common in cats, being encountered in 7.7% of the feline population (DiBartola *et al.*, 1987). Its prevalence increases with age reaching 15–30% in cats over 15 years old. Inherited forms have been documented in several breeds including Abyssinian, Persian, Siamese and Maine Coon (DiBartola *et al.*, 1987).

Experimental and clinical research has definitely established ACE as a key player in the progression of chronic kidney disease through persistent activation of the renin–angiotensin–aldosterone system as a maladaptive compensatory response to initial loss of renal function (Frohlich, 2001; Zaman *et al.*, 2002; Unger

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& Li, 2004; Chiurciu *et al.*, 2005). Administration of ACE inhibitor has been shown to slow the progression of chronic kidney disease in both humans and animals (Becker & Schölkens, 1987; Lefebvre & Toutain, 2004; King *et al.*, 2006).

The pharmacokinetic (PK) and pharmacodynamic (PD) properties of ramipril in cats have not been reported previously. This study examined (i) the plasma disposition and excretion of ramipril and ramiprilat in cats following oral administration, (ii) the PK and PD of ramipril and ramiprilat following oral administration of ramipril to healthy cats, (iii) the effect of feeding on the PK of ramipril in cats. Ramipril was administered as a single dose and once daily for nine consecutive days, at four different dose rates (0.125, 0.25, 0.5 and 1.0 mg/kg), to create steady-state conditions.

MATERIALS AND METHODS

Animals and design

Study 1: Pharmacokinetics and pharmacodynamics of ramipril

Twenty healthy European shorthair cats were randomly allocated to four treatment groups ($n = 5$ per group). Each treatment group received a single oral dose followed by repeated oral doses of ramipril (Vasotop[®] 1.25 mg and/or Vasotop[®] 2.5 mg and/or Vasotop[®] 5 mg tablets, Intervet), at dose rates of 0.125 mg/kg (group 1), 0.25 mg/kg (group 2), 0.5 mg/kg (group 3) and 1.0 mg/kg (group 4) body weight, once daily for nine consecutive days. There was a wash-out period of 13 days between the single- and repeated-dose studies.

Study 2: Effect of feeding on pharmacokinetics of ramipril

Eight healthy European shorthair cats were allocated at random to two treatment groups with two male and two female cats in each group. A two-period cross-over design with a washout period of 5 days was used. A single oral dose of ramipril (Vasotop[®] 1.25 mg tablets, Intervet), at a dose rate approximately 0.25 mg/kg body weight, was administered to fed (treatment 1) or fasted (treatment 2) cats. For treatment 1, cats were fed within approximately 0.5 h of tablet administration following fasting for at least 10 h. For treatment 2, cats were fed within approximately 4 h of tablet administration following fasting for at least 10 h.

Study 3: Excretion of ramipril

Three healthy male cats received a single oral dose of [¹⁴C]-ramipril (synthesized by Aventis Germany; radio-specific activity: 5075 MBq/g, radiochemical purity $\geq 97.95\%$) at a dose of 0.25 mg/kg body weight by oral gavage. The study was conducted by Avogadro, Fontenilles, France.

Sample collection

Study 1: Pharmacokinetics and pharmacodynamics of ramipril in cats

A jugular catheter was placed in each cat on the day before the first administration of ramipril in the single-dose study and on

the last day of the repeated-administration study. Other blood samples were taken by jugular venipuncture.

Blood samples were collected 5 days before and just before (0 h) and 0.5, 1, 2, 4, 6, 8, 12, 24, 36, 48 and 72 h after the single dose. For the repeated dose, blood sampling was performed before (0 h on days 1–9), and 0.5, 1, 2, 4, 6, 8, 12, 24, 36, 48 and 72 h after treatment (on day 9). Approximately 1.5 mL of blood was collected at each time-point and placed into clean, dry tubes without anticoagulant. The samples were centrifuged at around 2000 *g* at +4°C for 10 min. Serum was stored at –80°C until analysis.

Study 2: Effect of feeding on pharmacokinetics of ramipril in cats

Blood samples were collected just before and at 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48 and 72 h after treatment. Approximately 1.5 mL of blood was collected at each time-point and immediately transferred into lithium-heparin tubes. The samples were then centrifuged at around 2000 *g* at +4°C for 10 min. The resultant plasma was stored at –20°C until analysis.

Study 3: Excretion of ramipril in cats

Urine, faeces and cage washes were collected before and then at regular time intervals for 168 h after administration of [¹⁴C]-ramipril.

Assays

Ramipril and ramiprilat in serum

Ramipril and ramiprilat were quantified using high liquid chromatography coupled with positive electrospray tandem mass spectrometry (LC/MS/MS) with enalapril as the internal standard. The method was cross-validated in cat serum based on the method developed in cat plasma. The intra-day coefficients of variation (CV) varied from 1.9 to 4.2% for ramipril and from 2.4 to 5.3% for ramiprilat, respectively. The inter-day CV varied from 1.9 to 4.8% for ramipril and 2.4 to 6.3% for ramiprilat, respectively. The accuracy ranged from 4 to 7% for ramipril and from –1.0 to 0.7% for ramiprilat. The limits of quantification (LOQ) were 0.49 ng/mL for both ramipril and ramiprilat. The limits of detection (LOD) were 0.08 ng/mL and 0.05 ng/mL for ramipril and ramiprilat, respectively.

ACE activity

The determination of ACE activity was performed using a commercial radio-enzyme assay kit (Bühlman Laboratories, Basel, Switzerland). The assay works on a principle based on the ability of ACE to cleave the synthetic substrate, ³H-hippuryl-glycine-glycine into ³H-hippuric acid and a dipeptide. After acidification, the tritiated hippuric acid was separated from the unreacted substrate by extraction with scintillation cocktail and measured in a beta-counter. The limit of quantification was 7.43 units and the limit of detection was 1.05 units, the coefficients of variation for within-day and between-day precision varied from 3.0 to 3.2% and from 5.8 to 6.3%, respectively.

Determination of radioactivity

Duplicate aliquots of urine (approximately 200 μL), were accurately weighed, mixed with Ultima Gold (Perkin Elmer, Courtaboeuf, France) and stirred vigorously prior to liquid-scintillation counting. Samples of faeces were homogenized with Milli Q⁺ water (1:3, v/v) using a mixer. One millilitre of Solvable (Perkin Elmer, Courtaboeuf, France) was added to aliquots. The specimens were then dried at approximately 60°C both before and after the addition of isopropanol (1 mL). After cooling, the specimens were discoloured by the addition of 30% hydrogen peroxide and stirred gently. The specimens were then placed at room temperature for 10–30 min and 10 N hydrochloric acid was added. After stirring, Ultima Gold was added and the specimens were stirred again vigorously. The specimens were placed at approximately +5 °C prior to liquid scintillation counting. Duplicate aliquots of cage washes (approximately 1 mL) were mixed with Ultima Gold and stirred vigorously prior to liquid scintillation counting.

The total radioactivity of the different specimens was determined using a tri-Carb 2100 TR liquid scintillation counter (Perkin Elmer, Courtaboeuf, France) equipped with an automatic external standard quench correction (tSIE). Counting was carried out with 1% precision. For the different matrices (urine, faeces and cage washes), radioactivity background was determined on predose specimens (in duplicate) for each animal. The background value was the mean of the two determinations for each matrix from each animal. Blank specimens were treated as described above.

Limits of quantification was determined as a value twice equal to that of the background count of the pretreatment specimens. Values less than twice the background value were considered to be insignificant and recorded as below the LOQ. These values were considered equal to zero when calculating the mean. If the percentage difference in the radioactivity measurement between the two aliquots was greater than 10%, the assay was, where possible, repeated on two new aliquots and the new values used. The individual values reported were the average of two values.

Data analysis

Pharmacokinetic parameters

Study 1: Values below the LOQ of the assay (0.5 $\mu\text{g/L}$) were set at 0 $\mu\text{g/L}$ for calculation of the PK parameters. Observed values were used for maximum plasma concentration (C_{max}), trough plasma concentration ($C_{24\text{ h}}$) and time to maximum plasma concentration (t_{max}). The area under the plasma concentration vs. time curve for 24 h ($AUC_{0-24\text{ h}}$) and the entire treatment period ($AUC_{0-\text{tlast}}$) were calculated using the linear trapezoid rule without extrapolation to infinity. The apparent elimination half-life ($t_{1/2\beta}$) was also calculated. The accumulation ratio of ramiprilat was determined using the ratio of the $AUC_{0-24\text{ h}}$ with repeated doses to the $AUC_{0-24\text{ h}}$ following a single dose. Similar methodology was used for C_{max} and $C_{24\text{ h}}$. An estimate of the time to attain steady-state

was prepared by comparing $C_{24\text{ h}}$ values (individual and mean).

Study 2: Observed values were used for C_{max} and t_{max} . $AUC_{0-\text{tlast}}$ was calculated for ramipril and ramiprilat using AUC; this in turn was calculated using the trapezoidal rule (WinNonlin, version 3.2; Pharsight Corporation, Mountain View, CA, USA).

Pharmacodynamic parameters

Study 1: Serum ACE activity at each time-point was expressed as units of ACE activity. The percentage of inhibition of ACE activity (%) was calculated using the initial serum value (ACE activity before treatment) as a reference:

$$\text{ACE activity (\%)} = \frac{\text{ACE activity}}{\text{reference value}} \times 100 \quad \text{if ACE activity} \leq \text{reference value}$$

$$\text{ACE activity (\%)} = 100 \quad \text{if ACE activity} > \text{reference value}$$

The reference value for each cat was the average ACE activity in the samples taken before treatment (0 h in the single- and repeated-dose studies). Maximum effect (E_{max}), effect at 24 h ($E_{24\text{ h}}$), effect at 72 h ($E_{72\text{ h}}$), time of E_{max} (TE_{max}) and the area under the inhibition vs. time curve (AUC_{0-t}) were calculated following single and repeated doses of ramipril. Values below the LOD (1.05 units) were considered as zero. Values between the LOD and the LOQ (7.43 units) were taken into account for the calculation of the percentage of inhibition.

Statistical analysis

For the PK parameters, data are presented as geometric mean \pm CV for C_{max} , $C_{24\text{ h}}$, $AUC_{0-24\text{ h}}$ and $AUC_{0-\text{tlast}}$ (log-normal distribution). Data are presented as harmonic mean (range) for $t_{1/2\beta}$ and as median (range) for t_{max} . For the PD parameters, data are presented as geometric mean \pm CV% for E_{max} , $E_{24\text{ h}}$, $E_{72\text{ h}}$ and TE_{max} and as arithmetic mean \pm SD for AUC_{0-t} (normal distribution). Statistical analysis was performed utilizing the SAS[®] software (release 8.02; SAS Institute, Nantes, France). The level of significance (alpha) for each test was set at 0.05.

Study 1

AUC , C_{max} , $C_{24\text{ h}}$ and t_{max} for ramiprilat and ramipril (when possible) were compared between the treatment groups, using ANOVA followed by two-sided t -test for independent groups, and between the single-dose and the repeated-dose studies for each treatment group, using a two-sided t -test for paired observations. $E_{24\text{ h}}$, $E_{72\text{ h}}$, E_{max} and AUC_{0-t} were compared between the different treatment groups and between the single- and repeated-dose studies for each treatment group. An estimate of the time to attain steady-state was prepared by comparing $E_{24\text{ h}}$ values (individual and mean) using ANOVA for repeated measures. The statistical model comprised the dosage group as the effect arising out of the interaction between subjects and time, as well as the time-dose rate interaction as

the effect within subjects (using the Greenhouse–Geisser adjustment).

Study 2

C_{max} , t_{max} and $AUC_{0-t_{last}}$ for ramipril and ramiprilat were compared between fasted and fed cats by mixed ANOVA with feeding status, sequence, subject within sequence and period as factors. The effect of food on t_{max} was evaluated using the Wilcoxon signed rank test (SAS Version 8.1).

RESULTS

Pharmacokinetics of ramipril

The serum concentrations of ramipril are shown in Fig. 1. The PK parameters calculated after single and repeated doses of ramipril to cats are given in Table 1. C_{max} was attained rapidly [t_{max} 30 min (first sampling point) in most cats]. t_{max} was independent of the dosage regime (single or repeated dose).

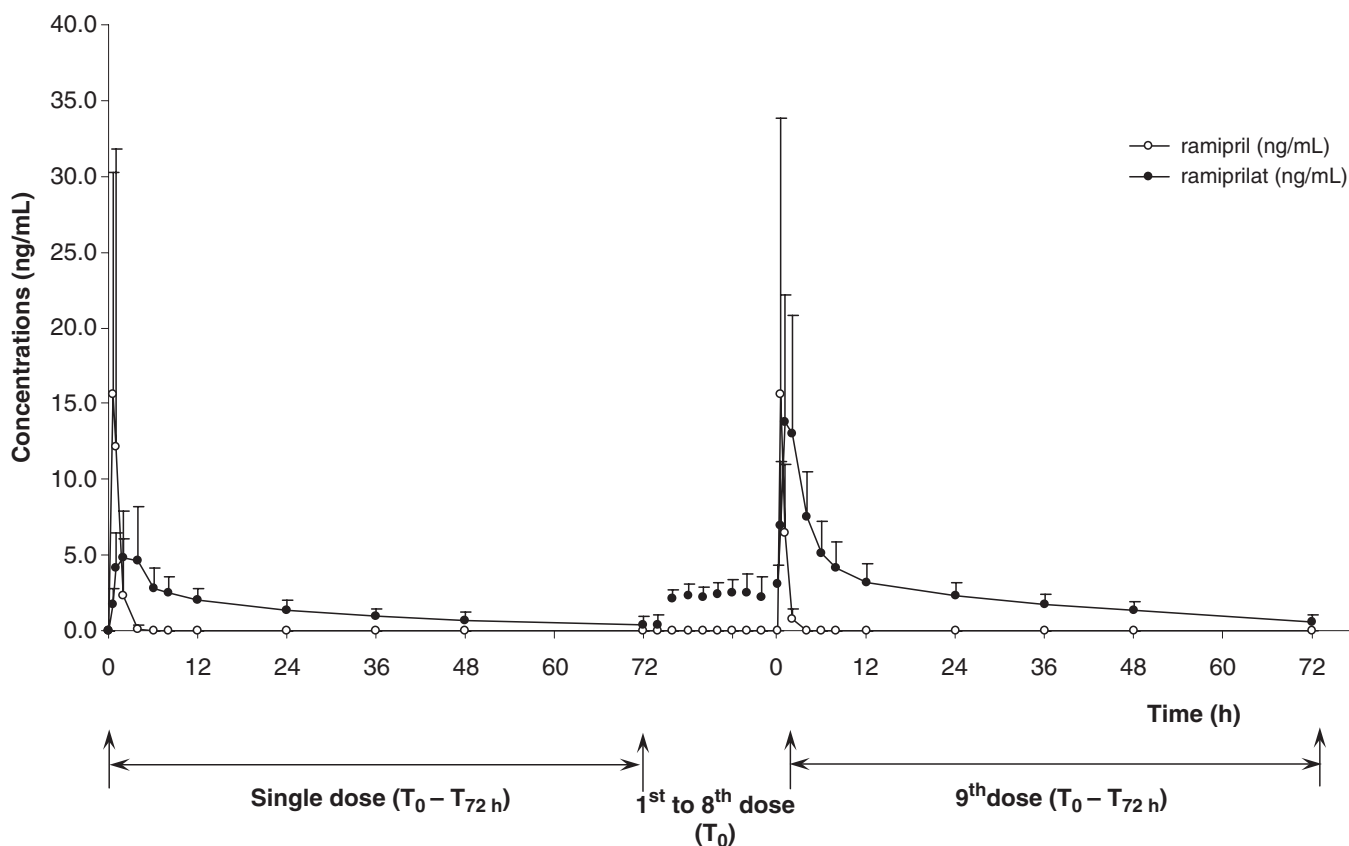


Fig. 1. Serum ramipril and ramiprilat concentrations (mean \pm SD) following single and repeated oral doses, at approximately 0.125 mg ramipril/kg, in healthy cats ($n = 5$ per dose group).

Table 1. Pharmacokinetics of ramipril in healthy cats following single and repeated oral doses (geometric mean \pm CV unless otherwise stated)

Parameters	Group 1 ($n = 5$)	Group 2 ($n = 5$)	Group 3 ($n = 5$)	Group 4 ($n = 5$)
	0.125 mg/kg	0.250 mg/kg	0.500 mg/kg	1.000 mg/kg
Single dose				
Dose rate (mg/kg)*	0.129 \pm 0.010	0.259 \pm 0.006	0.488 \pm 0.047	0.986 \pm 0.029
C_{max} (μ g/L)	12.27 \pm 22.2%	26.40 \pm 8.6%	53.96 \pm 4.4%	66.54 \pm 4.4%
t_{max} (h) [†]	0.5 (0.5–1)	0.5 (0.5)	0.5 (0.5–1)	1 (0.5–2)
$AUC_{0-t_{last}}$ (μ g·h/L)	11.21 \pm 24.9%	22.81 \pm 9.4%	47.85 \pm 5.0%	83.37 \pm 1.9%
Last dose (repeated dose study)				
Dose rate (mg/kg)*	0.135 \pm 0.015	0.264 \pm 0.018	0.478 \pm 0.055	0.966 \pm 0.026
C_{max} (μ g/L)	11.10 \pm 23.6%	17.94 \pm 16.7%	65.83 \pm 2.7%	108.52 \pm 1.9%
t_{max} (h) [†]	0.5 (0.5–1)	0.5 (0.5–4)	0.5 (0.5)	0.8 (0.5–4)
$AUC_{0-t_{last}}$ (μ g·h/L)	7.79 \pm 31.7%	14.54 \pm 17.4%	46.86 \pm 4.7%	141.39 \pm 1.1%

*Arithmetic mean \pm standard deviation.

[†]Median (range).

C_{\max} and AUC increased in a dose-dependent manner across the dose range tested (0.125–1.0 mg/kg). Ramipril concentrations declined rapidly following both single and repeated oral doses and were below the LOQ of the assay (0.49 ng/mL) 4 h after last treatment (range 2–12 h) (Fig. 1). No ramipril was detected in any of the pretreatment samples during the repeated dose study.

Pharmacokinetics of ramiprilat

The serum concentrations of ramiprilat are shown in Fig. 2. The PK parameters for ramiprilat calculated after single and repeated doses of ramipril to cats are given in Table 2.

Many of the PK parameters determined for the two lower dosages (0.125 and 0.25 mg/kg) were significantly different from those for the two higher dose rates (0.5 and 1 mg/kg). Peak ramiprilat concentrations were attained rapidly following administration. t_{\max} was independent of the treatment regime and also of the dosage regime. The C_{\max} and AUC of

ramiprilat increased linearly with the dose rate of ramipril administered. C_{\max} , C_{24h} , AUC_{0-24h} and $AUC_{0-tlast}$ were significantly higher following repeated-dose studies compared with single-dose studies at all four dosages tested ($P < 0.05$). The apparent elimination half-life was similar among the treatment groups and after single- and repeated-dose administration (Table 2). The accumulation ratio was higher at the lowest dose rate tested (0.125 mg/kg) than that at the other three higher doses, irrespective of whether it was calculated using AUC , C_{\max} or C_{24h} or after single- and repeated-dose administration (Table 2).

Pharmacodynamic parameters: determination of serum ACE activity

The mean serum ACE activity following single or repeated dose of ramipril is shown in Fig. 3. There was no difference between the treatment groups and between the single- and repeated-dose administrations with regard to the baseline serum ACE activity (data not shown). Serum ACE activity was below the

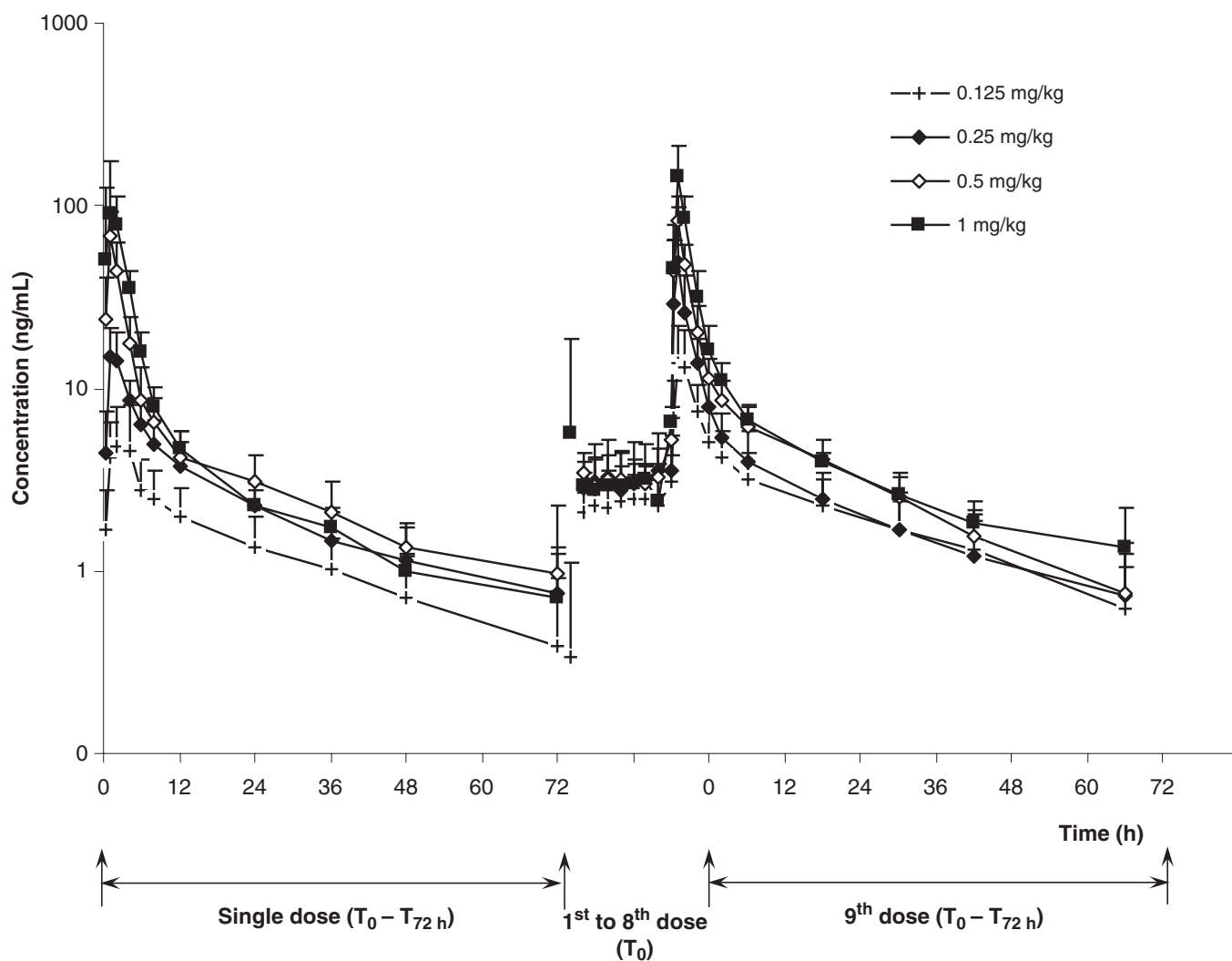


Fig. 2. Semi-logarithmic plot of serum concentrations of ramiprilat (mean \pm SD) following single and repeated oral doses of ramipril at 0.125, 0.25, 0.5 and 1.0 mg/kg in healthy cats ($n = 5$ per dose group).

Table 2. Pharmacokinetics of ramiprilat in healthy cats following single and repeated oral doses of ramipril (geometric mean \pm CV unless otherwise stated)

Parameters	Group 1 (n = 5)	Group 2 (n = 5)	Group 3 (n = 5)	Group 4 (n = 5)
	0.125 mg/kg	0.250 mg/kg	0.500 mg/kg	1.000 mg/kg
Single dose				
Dose rate (mg/kg)*	0.129 \pm 0.010	0.259 \pm 0.006	0.488 \pm 0.047	0.986 \pm 0.029
C _{max} (μ g/L)	4.68 \pm 39.6%	14.54 \pm 10.2%	66.93 \pm 2.1%	99.66 \pm 1.9%
t _{max} (h) [†]	2 (1–6)	2 (1–2)	1 (1–2)	2 (0.5–4)
C _{24h} (μ g/L)	1.24 \pm 126.7%	2.13 \pm 70.0%	2.89 \pm 48.8%	2.26 \pm 53.1%
t _{1/2β} (h) [‡]	29.23 (17.2–55.0)	28.65 (14.1–50.9)	25.65 (18.8–36.4)	21.30 (15.2–31.1)
AUC _{0–24h} (μ g·h/L)	52.07 \pm 3.1%	117.41 \pm 1.2%	242.14 \pm 0.0%	364.37 \pm 0.0%
AUC _{0–tlast} (μ g·h/L)	80.54 \pm 2.2%	173.95 \pm 0.8%	311.96 \pm 0.5%	421.73 \pm 0.3%
Last dose (repeated dose study)				
Dose rate (mg/kg)*	0.135 \pm 0.015	0.264 \pm 0.018	0.478 \pm 0.055	0.966 \pm 0.026
C _{max} (μ g/L)	12.89 \pm 12.8%	37.12 \pm 6.6%	79.06 \pm 1.8%	132.92 \pm 1.2%
t _{max} (h) [†]	1 (1–2)	1 (1–4)	1 (1)	1 (1)
C _{24h} (μ g/L)	2.14 \pm 71.0%	2.25 \pm 72.0%	3.91 \pm 34.0%	3.95 \pm 28.4%
t _{1/2β} (h) [‡]	28.68 (27.3–36.2)	22.76 (14.1–47.5)	20.33 (15.7–26.5)	31.54 (26.3–48.7)
AUC _{0–24h} (μ g·h/L)	103.21 \pm 1.5%	173.15 \pm 1.0%	311.54 \pm 0.4%	452.17 \pm 0.3%
AUC _{0–tlast} (μ g·h/L)	160.98 \pm 1.0%	224.89 \pm 0.8%	397.08 \pm 0.3%	559.76 \pm 0.2%
Accumulation ratio				
R-values (median)	Group 1	Group 2	Group 3	Group 4
AUC _{LD} /AUC _{SD}	1.91	1.51	1.32	1.37
C _{maxLD} /C _{maxSD}	2.62	1.94	1.11	1.77
C _{24hLD} /C _{24hSD}	1.74	1.13	1.40	1.68

*Arithmetic mean \pm standard deviation.[†]Median (range).[‡]Harmonic mean (range).

LOQ of the assay 30 min after the single-dose (range 2–4 h – except for two cats) or after repeated administration (range 1–6 h).

The PD parameters calculated following single or repeated dose of ramipril are given in Table 3. Following a single dose, E_{max} was significantly lower for the lowest treatment group (0.125 mg/kg) compared with the other three higher treatment groups ($P < 0.05$) (Table 3). Following administration of repeated doses, E_{max} was comparable in all four treatment groups (range E_{max}: 93.9–100.0%). TE_{max} was comparable between single and repeated doses for all the treatment groups. Dose-dependent inhibition of ACE activity was reported at 24 h (E_{24h}) in all cats. ACE activity was still inhibited 72 h (E_{72h}) after administration of ramipril. E_{72h} was significantly higher for the dose rate of 0.25 mg/kg between single and repeated dose ($P < 0.05$) but not for other dose rates. No T_{baseline} could be determined because the extent of inhibition lasted for more than 72 h, irrespective of the treatment group.

Routes of excretion

The results of the radio-labelled [¹⁴C]-ramipril are displayed in Table 4. The excretion of radioactivity was predominantly in faeces (86.8 \pm 1.9%) with only 10.7 \pm 1.3% recovered from urine and 0.9 \pm 0.6% from cage washes. This resulted in an excretion balance of 98.5 \pm 1.4% over 168 h, with approxi-

mately 80% of the dose having been excreted in faeces within 48 h following the administration.

Influence of feeding on the bioavailability of ramipril

The PK parameters of ramipril and ramiprilat in fed and fasted cats are summarized in Table 5. The food delayed t_{max} slightly but not significantly. There were no significant differences in C_{max}, C_{maxd}, AUC_{0–tlast} and AUC_{0–tlastd} for ramipril between fasted and fed cats. Ramipril concentrations declined rapidly and fell below the LOQ (0.49 ng/mL) at 3 h post administration in fasted (range 1–3 h) and fed (range 1–6 h) cats. There was no significant difference in C_{max}, t_{max}, C_{maxd}, AUC_{0–tlast} and AUC_{0–tlastd} for ramiprilat in fed and fasted cats ($P > 0.05$). Plasma concentrations of ramiprilat were above the LOQ until the last time-point 72 h in all of the cats (both fasted and fed).

DISCUSSION

Following oral dose to cats, ramipril is absorbed rapidly and converted into its active metabolite ramiprilat. In humans and in dogs, it has been shown that ramipril is converted into ramiprilat by de-esterification (hydrolysis) in the liver (Eckert *et al.*, 1984). This is also likely to occur in cats, as the disappearance of ramipril from serum coincides with the appearance of ramiprilat.

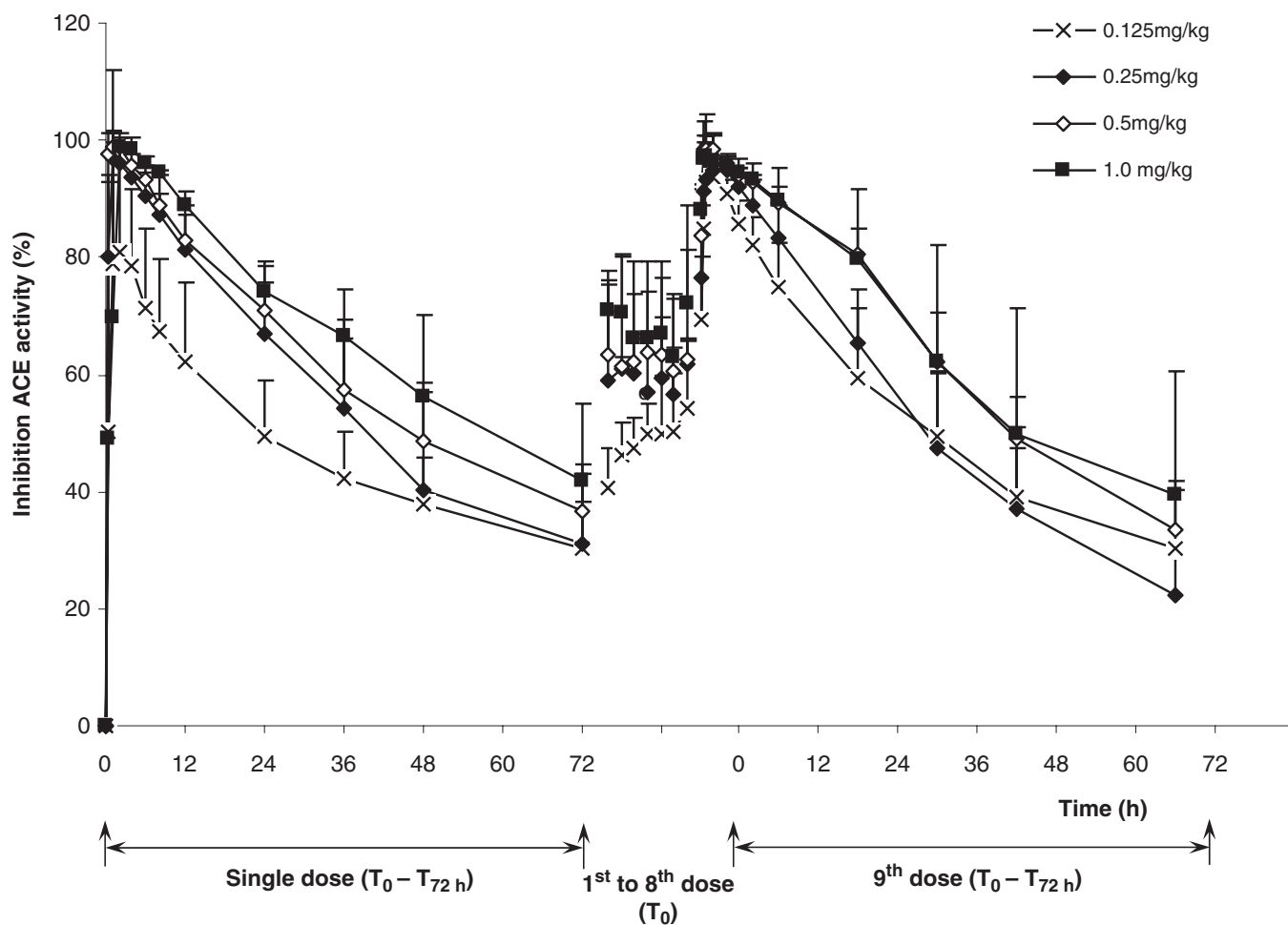


Fig. 3. Serum ACE activity (mean \pm SD) following single and repeated oral doses of ramipril at 0.125, 0.25, 0.5 and 1.0 mg/kg in cats ($n = 5$ cats per dose group).

Table 3. Serum ACE activity following single and repeated oral administration of ramipril to healthy cats ($n = 5$ per group)

Parameters median (range)	Group 1	Group 2	Group 3	Group 4
	0.125 mg/kg	0.250 mg/kg	0.500 mg/kg	1.000 mg/kg
Single dose				
E_{max} (%)	89.1 (65.2–94.4)	95.6 (94.6–100)	100.0 (95.6–100)	100.0 (96.9–100)
E_{24h} (%)	46.0 (39.3–63.8)	59.7 (57.4–83.1)	70.9 (64.6–77.1)	75.0 (65.6–79.3)
E_{72h} (%)	27.9 (22.4–39.5)	30.0 (15.0–46.3)	33.4 (28.8–46.8)	41.2 (26.5–57.0)
TE_{max} (h)	2.0 (1–6)	1.0 (1–2)	0.5 (0.5–1)	1.0 (0.5–2)
AUC_{0-t} (% inh·h)*	3349.8 \pm 632.0	4092.7 \pm 868.0	4431.7 \pm 425.8	4812.0 \pm 587.2
Last dose (repeated dose study)				
E_{max} (%)	93.9 (93.1–100)	96.6 (93.9–100)	100.0 (95.2–100)	97.1 (95.6–100)
E_{24h} (%)	54.7 (46.3–73.7)	61.6 (53.8–76.7)	81.1 (74.0–85.3)	82.6 (62.6–90.5)
E_{72h} (%)	28.5 (17.5–45.5)	23.5 (9.8–34.9)	31.8 (25.7–43.7)	40.5 (12.3–64.1)
TE_{max} (h)	1.0 (1–2)	1.0 (1–4)	0.5 (0.5–1)	0.5 (0.5–2)
AUC_{0-t} (% inh·h)*	3843.7 \pm 721.0	3869.2 \pm 610.0	4665.0 \pm 348.1	4736.9 \pm 1075.8

*Arithmetic mean \pm SD.

The frequency of blood sampling was limited in the initial hour after administration in this study. This prevented detailed description of the PK of ramipril. However, peak concentrations

of ramipril were attained rapidly (at or before 2 h) in most of the treated cats and returned within a fairly short period (4 h, range 2–12 h) to below the LOQ.

Table 4. Cumulative percentage of dose (mean \pm SD) after administration of [14 C]-ramipril to cats

Collection period (h)	Faeces		Urine		Cage washes		Total	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0–6	/	/	2.25	0.78	/	/	2.25	0.78
0–24	35.86*	–	5.69	3.47	0.64	0.68	30.24	44.19
0–48	80.34	7.06	9.33	0.80	0.76	0.63	90.43	7.04
0–72	86.11	2.09	10.09	1.25	0.83	0.61	97.03	1.63
0–96	86.36	2.01	10.39	1.30	0.86	0.60	97.61	1.46
0–120	86.65	1.90	10.54	1.30	0.88	0.60	98.07	1.40
0–144	86.72	1.93	10.62	1.25	0.93	0.60	98.27	1.35
0–168	86.80	1.93	10.74	1.29	0.94	0.61	98.48	1.36

* $n = 2$.**Table 5.** Pharmacokinetics of ramipril after oral administration of a single dose (0.25 mg/kg) to fasted and fed cats

Parameters (geometric mean \pm SD)	Fasted cats ($n = 8$)	Fed cats ($n = 7$)
Ramipril C_{max} (μ g/L)	16.97 \pm 1.94	15.91 \pm 1.71
Ramipril t_{max} (h)*	0.5 (0.5–1)	1.0 (0.5–2)
Ramipril $AUC_{0-t_{last}}$ (μ g·h/L)	13.14 \pm 1.97	13.87 \pm 1.70
Ramiprilat C_{max} (μ g/L)	12.25 \pm 2.03	9.57 \pm 1.97
Ramiprilat t_{max} (h)*	1.4 (1–1.8)	1.5 (1–6)
Ramiprilat $AUC_{0-t_{last}}$ (μ g·h/L)	136.5 \pm 1.3	130.8 \pm 1.3

*Median (range).

The rate and extent of absorption of ramipril as well as its conversion into ramiprilat were not significantly influenced by the presence of food in the gastrointestinal tract. This suggests that ramipril can be administered to cats at any time independent of the time of feeding.

Previous studies with the ACE inhibitors, like ramipril in dogs (Lefebvre *et al.*, 2006) or benazepril in dogs (Toutain *et al.*, 2000) and cats (King *et al.*, 2003), suggest that the bioavailability of ACE inhibitors is low. The bioavailability of ramipril could not be calculated in this study, as ramipril was not administered intravenously to cats. However, it is likely that ramipril has limited bioavailability (less than 5%) in cats and that most of this pro-drug either remains in the gastrointestinal lumen following oral administration or is bio-transformed by the liver into its active metabolite ramiprilat; as for the other ACE inhibitors, the elimination of ramipril occurs in two (or three) phases.

The apparent elimination half-life was the same over the dosage regime tested (0.125–1.0 mg/kg) and after single or nine repeated doses (approximately 25 h). This long terminal half-life would suggest that accumulation of ramiprilat may occur following repeated doses. This was confirmed by the accumulation ratio of 1.91 calculated for the lowest dose rate tested (0.125 mg/kg). However, the accumulation ratio appeared to be inversely correlated to the dose administered (1.91 for 0.125 mg/kg compared with 1.37 for 1.0 mg/kg). This is unusual, based on standard PK concepts for drugs with long terminal half-lives (Toutain & Bousquet-Mélou, 2004), but is

likely because of the nonclassical PK of ACE inhibitors (Toutain & Lefebvre, 2004). In this respect, the initial fast elimination phase (the distribution phase in classical compartmental models) is thought to reflect renal (or nonrenal) clearance of free ramiprilat from the circulation, whereas the long terminal phase (the elimination phase in classical compartmental models) can be attributed to the dissociation of ramiprilat from its binding site on free, circulating and tissue-bound forms of ACE before elimination from the body (Bünning, 1987; Toutain *et al.*, 2000; Toutain & Lefebvre, 2004). Thus, at the lowest dose rate tested (0.125 mg/kg), ramiprilat, because of its high affinity for ACE, saturates the ACE pool progressively up to the stage of saturation of the entire pool, which is achieved approximately after two administrations (steady-state). Consequently, ramiprilat accumulation is limited to the first two administrations and no further accumulation is likely to occur following subsequent administration of ramipril to cats.

ACE activity is almost completely abolished 0.5–2.0 h after ramipril treatment, irrespective of the dose tested (0.125–1.0 mg/kg) and significant inhibition of 54.7–82.6% (depending on the dose) was still present 24 h after treatment. Thus, oral administration of ramipril produces rapid and long-lasting inhibition of serum ACE activity in cats. The extent of inhibition is comparable with that achieved irrespective of the dosage administered. Maximal inhibition was attained with the lowest dose tested in this study (0.125 mg/kg). This suggests that the binding sites for ramiprilat on ACE become saturated at a dose rate as low as 0.125 mg ramipril/kg. Steady-state conditions for inhibition were attained as early as the second dose. Although E_{24h} was lower following 0.125 mg/kg, the AUC was not significantly different for the different dosages tested. This may indicate that the dose rate may be increased to the immediately higher one, i.e. 0.25 mg/kg in some animals to obtain maximal inhibition of ACE activity between two successive doses. This is already recommended for the treatment of canine congestive heart failure (NYHA grades II–IV) where the dose range of ramipril is 0.125–0.25 mg/kg.

Similar to other ACE inhibitors, ramiprilat binds ACE competitively and with high affinity (Bünning, 1987) and results rapidly in strong inhibition of ACE activity. Even at a dose rate of 0.125 mg/kg in normal cats, ACE inhibition was almost complete (94%) within 1–1.5 h and remained above 50% for 24 h, allowing once-daily dosing. Higher doses of ramipril (0.25–1.0 mg/kg) do not significantly alter the PD profile, except that ACE inhibition persists for longer, with more than 80% inhibition at 24 h and 40% at 72 h, similar to what has been shown for benazepril at 0.25–2.0 mg/kg (King *et al.*, 1999, 2002).

Exposure to ACE inhibitors that are mainly excreted via the kidney can markedly increase in patients suffering from renal insufficiency (Oguchi *et al.*, 1993; Lefebvre *et al.*, 1999). Ramipril is excreted at 40% in faeces and 56% in urine in humans (Becker & Schölkens, 1987) and a 25 to 50% reduction in the dose is a common practice in human patients suffering from renal impairment (Meisel *et al.*, 1994; Song & White, 2002).

In the excretion study, most of recovered radioactivity was found in faeces (87%) and to a lesser extent in urine (11%). This suggests that ramipril is excreted mostly by the faecal route in cats. However, no specific study was performed to determine the bioavailability of ramipril in cats after oral administration. In dog, the bioavailability of ramipril after oral administration is approximately 6–7% (Lefebvre *et al.*, 2006) and 79% of ramipril is excreted in faeces and 15% in urine (Becker & Schölkens, 1987). Whether the bioavailability of ramipril is as low as this in the cat remains to be confirmed.

The results of this study suggest that the rate and extent of excretion of ramipril would not be altered significantly in cats with mild to moderate chronic kidney disease. There is experimental support for this hypothesis in both dogs and cats. In an experimental model of surgically induced renal impairment in dogs, it was demonstrated that the PKs and PDs of ramipril, following repeated oral doses, remained unchanged compared with that in controls (Lefebvre *et al.*, 2006). That study concluded that no adjustment in dosage of ramipril was required in dogs with mild to moderate renal impairment (Lefebvre *et al.*, 2006). Similar findings have been reported for the ACE inhibitor benazepril, which is eliminated up to 85% in faeces, in cats (Brown *et al.*, 2001; King *et al.*, 2002). The latter study showed that there was no significant change in exposure to benazeprilat following oral administration of benazepril to healthy cats or to cats with surgically induced mild renal insufficiency (King *et al.*, 2002). Whether this is because of a relative independence of the clearance of the drug from renal function or on account of increased binding to ACE in renal insufficiency, as demonstrated for benazepril in dogs (Toutain *et al.*, 2000) and cats (King *et al.*, 2002), is not completely understood.

Ramipril at a dose rate of 0.125 mg/kg produces significant and long-lasting inhibition of ACE activity in healthy cats. Ramipril is mainly excreted in faeces in cats. As chronic kidney disease alters many physiological parameters, further studies are required to examine the effect of chronic kidney disease on the PK and PD of ramipril in cats.

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