

Pharmacokinetic and Pharmacodynamic Parameters of Ramipril and Ramiprilat in Healthy Dogs and Dogs with Reduced Glomerular Filtration Rate

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Ramipril, an angiotensin-converting enzyme (ACE) inhibitor for use in dogs, is converted *in vivo* to its active form, ramiprilat, which is eliminated in the bile and urine in the dog. The objective of this study was to assess the effect of renal impairment on the pharmacokinetics (PKs) and pharmacodynamics (PDs) of ramipril and ramiprilat. Ten adult Beagle dogs were used. PK/PD studies were performed before and after the induction of subclinical renal impairment. Ramiprilat was given at 0.25 mg/kg by a single IV bolus. After a 2-week washout period, ramipril was administered PO at 0.25 mg/kg once daily for 8 days. Ramipril and ramiprilat PKs were studied by using a physiologically based model. The relationship between free plasma ramiprilat concentration and ACE activity was described by using the fractional Hill model. Glomerular filtration rate was decreased by 58%. No biologically relevant changes in usual plasma variables were observed between the 1st and the 8th day of oral treatment with ramipril under either condition. After an IV bolus of ramiprilat, the only changes in renal-impaired dogs were a 14 and 49% decrease in clearance of the free fraction of ramiprilat ($P < .01$) and free plasma concentration required to produce 50% of the maximal effect ($P < .05$), respectively. After repeated PO administration of ramipril, there were no alterations in any of the PK and PD parameters in healthy or renal-impaired dogs. No adjustment of the recommended PO dosage of ramipril is needed in dogs with moderate renal impairment.

Key words: Angiotensin; Angiotensin-converting enzyme inhibitors; Kidney; Modeling; Renal dysfunction.

Ramipril is a prodrug that undergoes de-esterification to form ramiprilat, a nonsulfhydryl angiotensin-converting enzyme (ACE) inhibitor (ACEI). Most of this metabolic transformation is considered to occur in the liver. In the United States and Europe, ramipril has been used in human medicine for the treatment of heart failure and renal disease since the 1980s and has been recently approved in Europe for the treatment of heart failure in dogs. Ramipril was shown to normalize plasma and left ventricle angiotensin II concentrations in dogs with experimental mitral regurgitation,^{1,2} to increase nitric oxide production in canine coronary microvessels and to reduce myocardial oxygen consumption,³ and to prevent associated pulmonary congestion and hypertension in dogs with experimental mitral valve insufficiency.¹ Data also have been published about its clinical efficacy in the treatment of dogs with naturally acquired heart failure.⁴

In contrast to humans,⁵ no information was available in the literature about the pharmacokinetic (PK) and pharmacodynamic (PD) properties of ramipril in dogs, except 1 PK study with radiolabeled ramipril performed 20 years ago in 3 dogs.⁶ The PK and PD properties of enalapril, benazepril, and their active metabolites have been documented in dogs in several studies.^{7–13} These investigations have provided not only PK parameters, knowledge of which, as for any drug, is required for

adequate drug prescription, but also have contributed to the development of an original PK/PD modeling approach allowing appropriate interpretation of the plasma profile¹² (see later). The disease condition for which the impact on ACEI disposition has been studied most extensively in humans and dogs is renal failure. Renal dysfunction may occur in dogs with heart failure, and therefore assessment of the influence of renal impairment on ACEI disposition is needed. Many ACEIs in humans are cleared mainly or totally by the kidneys and overexposure may occur in patients with renal impairment. In dogs with experimental renal impairment, such overexposure has been demonstrated for enalaprilat.^{11–13} The potential adverse effects of ACEI, the risk of occurrence of which may increase in patients with renal dysfunction, include 1st-dose acute hypotension, acute renal failure, and hyperkalemia.¹⁴

The aim of the present study was to determine PK and PD parameters of ramipril and ramiprilat administered by PO and IV routes, respectively, in the healthy dog; to evaluate the effect of renal impairment on the PK and PD properties of this drug; to propose dosage regimen adjustment, if needed; and to assess the effect of ramipril treatment on plasma biochemical variables in healthy and renal-impaired dogs.

Materials and Methods

Animals

Ten female Beagle dogs aged 4–6 years and weighing 10.4–14.4 kg were used. All animals were healthy based on a complete physical examination and plasma biochemistry. The animals were housed individually in an air-conditioned room. Water was given *ad libitum*. A commercial diet^a for healthy adult dogs was offered (approximately 250 g/d) once daily at 12:00 PM throughout the study. The dogs were acclimatized to the experimental conditions for 1 month before the 1st phase of the study. The study was performed according to the National Institutes of Health *Guide for Care and Use of Laboratory Animals*.

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Experimental Design

Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were 1st (at day 0) assessed by using iohexol and *p*-aminohippuric acid (PAH) plasma clearances, respectively, after IV administration of the test substances under control conditions. One week later (at day +8), IV bolus administration of ramiprilat was performed. Two weeks later (at day +23), ramipril was administered PO once daily for 8 days (up to day +30). After 3 months (from day +120 to day +125), moderate renal impairment (reduction of normal GFR by approximately 60%) was experimentally induced by surgery. Afterwards, iohexol and PAH (at day +140), ramiprilat (at day +148), and ramipril (at day +163) PKs were performed as for the control conditions.

Model of Renal Impairment

Experimental renal impairment was carried out, as described previously.¹⁵ The 24 hour-fasted dogs were pretreated with acepromazine maleate^b (0.1 mg/kg IM) 30 minutes before induction of general anesthesia with sodium thiopental^c (15 mg/kg IV). Anesthesia was maintained with isoflurane^d (2% vol/vol) after endotracheal intubation, with an oxygen flow rate of approximately 1.5 L/min. Briefly, renal impairment was induced by using a nephrectomy-electrocoagulation procedure, performed in dorsal recumbency. The abdomen was entered through a midline incision. The left kidney was exposed by packing the abdominal viscera behind the mesentery of the descending colon. The right kidney was exposed by using the mesoduodenum in a similar fashion and retracting the viscera. The right kidney was freed from its sublumbar attachment by a combination of sharp dissection with scissors and blunt digital dissection. The renal artery, vein, and ureter were isolated successively and ligated separately before excision of the kidney. Portions of the left renal cortex were electrocoagulated by repeated 1-cm stabs with a stainless steel probe connected to an electrocautery unit. The number of stabs per kidney (120) was sufficient to induce moderate renal impairment (ie, approximately 60% of normal GFR). Hemorrhage was controlled by manual pressure on the kidney.

Morphine hydrochloride^e was administered IV twice, just after induction (5 mg) and just after surgery (5 mg); and cephalixin^f (15 mg/kg) was administered IV at induction. The surgical wound was inspected and rectal temperature was measured every morning for 5 days after surgery.

Biochemistry

Blood samples (2 mL) were taken from the left jugular vein just before the beginning of each kinetic study for the determination of plasma biochemistry parameters. Blood was placed in heparinized tubes and centrifuged (3,500 × *g*, 10 minutes, 4°C). Plasma was stored at -20°C until analysis. The following plasma biochemistry parameters were determined using an analyzer^g: sodium, potassium, chloride, bicarbonate, phosphorus, total proteins, aspartate aminotransferase, alanine aminotransferase (ALT), blood urea nitrogen, and creatinine.

GFR and ERPF Assessment

Glomerular filtration rate and ERPF were determined by using plasma clearance of iohexol and PAH,^h respectively, as described previously.¹⁶ The PAH solution was prepared with sterile 0.9% sodium chloride to obtain a final concentration of 100 mg/mL and administered at a nominal dosage of 10 mg/kg. Iohexol, as an injectable solution of 647 mg/mL,ⁱ was administered IV simultaneously with PAH via an indwelling catheter placed in the right cephalic vein, at a nominal dosage of 64.7 mg/kg.

One milliliter of blood was directly sampled from the jugular vein, placed in a heparinized tube, and centrifuged (3,500 × *g*, 10 minutes, 4°C). Three 0.15-mL-aliquots of plasma were stored at -20°C. Samples were obtained before (*t*₀) and at 2, 5, 10, 20, 30, 60, and 90 minutes, and at 2, 3.5, 6, and 10 hours after administration.

Ramiprilat and Ramipril PK Studies

Ramiprilat solution (4-mL vial of ramiprilat at 1 mg/mL)^j was administered IV, at a dosage of 0.25 mg/kg, via an indwelling catheter placed in the right cephalic vein. For PO administration, ramipril tablets^k were administered at a dosage of 0.25 mg/kg once a day (9:30 AM) for 8 consecutive days. The tablets were administered inside a aliquot (about 5 g) of pet food. The number of tablets of different strengths (1.25 and 2.5 mg) was adjusted to the body weight of the dog.

Blood samples were taken before (*t*₀) and at 2, 8, 15, and 30 minutes, and at 1, 2, 4, 6, 8, 10, 24, 48, and 72 hours after the IV bolus of ramiprilat. During the oral PK study, blood samples were taken before (*t*₀) and after test substance administration according to the following time schedule: day 1: at 0 (just before administration), 15, and 30 minutes, and 1, 2, 4, and 8 hours; days 2 and 3: at 0 (just before administration) minutes and 2 hours; days 4 and 5: at 0 (just before administration) minutes; and day 8: at 0 (just before administration), 15, and 30 minutes, and 1, 2, 4, 8, 24, 48, 72, and 120 hours. For each sampling time, 1.5 mL of blood was taken from the left jugular vein and placed in a heparinized tube and centrifuged (3,500 × *g*, 10 minutes, 4°C) and aliquots of plasma were stored at -20°C until assay.

Assays

Plasma iohexol and PAH concentrations were determined as previously described.¹⁶ Ramipril and ramiprilat were quantified in plasma by using a validated liquid chromatography coupled with positive electrospray tandem mass spectrometry (LC-MS-MS) method (Intervet file, unpublished date). Enalapril was used as the internal standard. The LC-MS-MS consisted of an Alliance 2690^l and a Quattro liquid chromatograph.^m The analytes were separated by chromatography using symmetry C8 preceded by a guard column. Gradient methods were used with 2 solvents: ammonium acetate 0.2 mM-formic acid 0.1%/methanol (80/20 vol/vol) for mobile phase A and ammonium acetate 0.2 mM-formic acid 0.1%/acetonitrile (10/90 vol/vol) for mobile phase B. The flow rate was 0.4 mL/min. The mass spectrometer was operated in positive electrospray ionization mode with multiple reaction monitoring. Plasma samples (0.2 mL) were purified by using an automated solid-phase extraction using STRATA SDB-L cartridges.ⁿ After evaporation to dryness, the residue was injected into the LC-MS-MS system.

The intraday and interday coefficients of variation for the LC-MS-MS assay of ramipril and ramiprilat in plasma were <6% and 9%, respectively. Accuracy data ranged from -2 to 7%. The assay system provided limits of quantification of 0.4 ng/mL for both ramipril and ramiprilat.

Plasma ACE activity was measured with a commercial radioenzymatic assay (REA) kit.^o The assay depends 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 is separated from unreacted substrate by extraction with scintillation cocktail and measured in a beta counter. This REA method was validated; the limit of quantification was 2.08 units, and the coefficients of variation for within-day and between-day precision were 3.1 and 5.2%, respectively.

PK and PD Analysis

Data for iohexol and PAH were subjected to noncompartmental analysis using a statistical moment approach with WinNonLin.⁹ Plasma clearances were calculated by dividing the dose administered by the area under the plasma concentration versus time curve (AUC). AUC was determined using the linear trapezoidal rule with extrapolation to infinity. The decrease (D, %) in GFR (or ERPF) was determined using the following equation:

$$D = \left(\frac{Cl_e - Cl_p}{Cl_e} \right) \times 100$$

where Cl_e and Cl_p are the plasma exo-iohexol (or PAH) clearance before and after surgery, respectively.

The PK and PD data for ramipril and ramiprilat were analyzed as described previously.^{12,17} Briefly, a nonconventional monocompartmental model was constructed assuming, as for all investigated ACEIs, that unbound drug is the sole form eliminated with a rate constant K_{10} (1/h) from the central compartment with volume V_c (L/kg). Ramiprilat binds specifically and saturably to ACE (high affinity and low capacity) and nonsaturably to plasma albumin (low affinity and high capacity). ACE consists of both circulating and tissue components; thus, ramiprilat concentrations measured in plasma correspond to the sum of free ramiprilat, ramiprilat specifically bound to circulating ACE, and ramiprilat bound nonspecifically to albumin. In contrast, ramiprilat bound to tissue ACE is not measured by the assay. For modeling purposes, all of the ACE was assumed to be located in the central sampling compartment (for details and explanations, see review by Toutain and Lefebvre¹⁷). Because circulating ACE originates from tissue ACE, the circulating and noncirculating forms of ACE share the same binding parameters: B_{max} (nmol/kg), which is the maximal binding capacity, and K_d (nM), which is the equilibrium dissociation constant (ie, the free plasma ramiprilat concentration corresponding to half-saturation of the entire ACE pool). The circulating fraction (f_{circ}) of ACE, from 0 to 1, was estimated as a parameter of the model given the sharing of binding capacity between circulating ACE (ie, $f_{circ} \times B_{max}$) and tissue ACE (ie, $(1 - f_{circ}) \times B_{max}$). B_{max} and K_d were estimated in terms of amount (nmol/kg) but were expressed in terms of concentration (nM) by dividing the estimated amount by the volume of distribution (L/kg) of the free fraction (ie, V_c). The plasma clearance (Cl, mL/kg/min) of free ramiprilat was determined by multiplying K_{10} by V_c . The bioavailability of ramiprilat after PO administration of ramipril (F; ie, the systemic bioavailability of ramiprilat after PO administration of ramipril) was calculated for each dog from the ratio of Cl after IV administration of ramiprilat and from Cl/F after PO administration of ramipril. The binding capacity of nontissue ACE (P_{max} , nM) was determined by multiplying B_{max} by f_{circ} . Additional details are provided by Toutain et al¹² and the corresponding equations are given by Toutain and Lefebvre.¹⁷

The relationship between the plasma concentration of free ramiprilat and ACE activity was described by the fractional Hill model according to the following equation:

$$E(t) = E_0 \left(1 - \frac{C^n}{IC_{50}^n + C^n} \right)$$

where $E(t)$ is the measured plasma ACE activity at time t , E_0 is the control ACE activity value; C is the plasma concentration of free ramiprilat, IC_{50} is the plasma concentration of free plasma ramiprilat required to produce 50% of the total inhibition of ACE, and n is a power term representing the steepness of the concentration-effect curve. Additional details are provided by Toutain et al.¹²

Statistical Analysis

Statistical analyses were carried out using Systat.⁴ Results are expressed as mean \pm standard deviation.

Statistical analyses of the effect of renal impairment on each of the PK and PD parameters of ramiprilat were carried out using the following general linear model:

$$Y_{i,j} = \mu + D_i + K_j + \varepsilon_{ij}$$

where, $Y_{i,j}$ is the value observed for dog i in a given condition j , μ is the general mean effect, D_i is effect of dog i ($i = 1-10$), K_j is renal status of the animal ($j = 1$ [healthy] or 2 [renal-impaired]), and ε_{ij} is error of the model. A similar model was used to study the effect of renal impairment on body weight and biochemical variables.

To test the effect of oral ramiprilat treatment for 8 consecutive days on the biochemical variables in a given condition (healthy or renal-impaired), the following general linear model was used:

$$Y_{i,j} = \mu + D_i + P_j + \varepsilon_{ij}$$

where, $Y_{i,j}$ is the value observed for dog i on a given day of the treatment, μ is the general mean effect, D_i is effect of dog i ($i = 1-10$), P_j is day of the treatment ($j = 1$ [start of the treatment] or 2 [end of the treatment]), and ε_{ij} is error of the model. For all analyses, the level of significance (α) was set at 0.05.

Results

Clinical and Biochemical Findings

All dogs recovered rapidly after surgery. No painful reaction was observed when handling the animal and palpating the surgical wound on the days after surgery. Impairment of renal function induced a slight but significant decrease ($P < .001$) in body weight (11.8 ± 1.16 and 11.4 ± 1.12 kg in the pre- and postoperative periods, respectively), but there was no apparent related change in appetite. A significant increase in plasma sodium, chloride, bicarbonates, urea, creatinine, and ALT concentrations was observed (Table 1). However, these changes, except for urea and creatinine, were very mild. Ramipril and ramiprilat treatment was well tolerated in all animals under both conditions.

Effect of Induction of Renal Impairment on GFR and ERPF Values

The exact dosages of PAH and iohexol were 9.7 ± 0.3 and 66.0 ± 2.6 mg/kg, respectively. Surgery induced a significant decrease ($P < .001$) in GFR and ERPF. The mean values of GFR were 3.8 ± 0.56 and 1.6 ± 0.26 mL/kg/min before and after induction of renal impairment, respectively. The corresponding values of ERPF were 12.5 ± 3.19 and 4.9 ± 1.20 mL/kg/min. The mean \pm standard deviation (SD) decrease in GFR and ERPF was 58 ± 8.3 and $59 \pm 13.0\%$, respectively.

Effect of Repeated PO Administration of Ramipril on Plasma Biochemical Variables

Between the 1st and the 8th PO administration of ramipril, a statistically significant, but very slight, increase was observed for plasma sodium (from 140 ± 1.3 to 143 ± 1.4 mEq/L, $P < .001$) and chloride (from 109 ± 2.3 to 111 ± 2.6 mEq/L, $P < .01$) concentrations

Table 1. Biochemical plasma variables in 10 adult Beagle dogs under healthy and renal-impaired conditions.

Variable	Condition	
	Healthy	Renal-Impaired
Sodium (mEq/L)	141 ± 1.5	144 ± 1.8***
Potassium (mEq/L)	3.8 ± 0.32	4.0 ± 0.66
Chloride (mEq/L)	110 ± 2.3	113 ± 2.3***
Bicarbonates (mEq/L)	17.7 ± 1.60	19.4 ± 1.57***
Total proteins (g/L)	60 ± 4.0	59 ± 3.4
Blood urea nitrogen (mg/dL)	12 ± 2.0	26 ± 7.5***
Creatinine (mg/dL)	0.7 ± 0.10	1.4 ± 0.26***
Phosphorus (mg/dL)	3.4 ± 0.71	3.4 ± 0.59
Asparatase		
aminotransferase (U/L)	26 ± 3.7	25 ± 4.5
Alanine		
aminotransferase (U/L)	26 ± 12.9	35 ± 15.7**

** $P < .01$; *** $P < .001$.

in healthy conditions and plasma bicarbonate (from 19.2 ± 1.40 to 19.9 ± 1.52 mEq/L, $P < .05$) concentration under renal-impaired conditions.

PK and PD Parameters

The exact dosages of ramiprilat and ramipril were 0.253 ± 0.003 mg/kg and 0.258 ± 0.014 mg/kg, respectively. The mean \pm SD plasma concentration versus time profiles are presented in Figures 1 and 2. After PO administration of ramipril, the test substance was rapidly absorbed and transformed into ramiprilat so that the plasma ramipril concentration was below the limit of quantification for most sampling times, except at 15 and 30 minutes after dosing. For this reason, the ramipril data were not analyzed. Visual inspection shows that the plasma concentration versus time curves of ramiprilat under both conditions are essentially superimposable.

Figures 3 and 4 present the mean \pm SD of the inhibition effects of ramiprilat on the ACE activity in the 10 dogs. Mean \pm SD of the PK and PD parameters of ramiprilat after administration of ramiprilat and ramipril are given in Tables 2 and 3. ACE activity was totally abolished in all dogs 2 minutes after administration of ramiprilat, and up to 2 and 4 hours, respectively, under healthy and renal-impaired conditions. Three days after IV administration, ACE activity was 82 ± 7 and $74 \pm 6\%$ of the pretreatment value under healthy and renal-impaired conditions, respectively. After PO administration, ACE inhibition showed larger interindividual variability in the 1st hours. Two hours after the 8th PO administration under healthy conditions, ACE activity was minimal; it was abolished in 7 dogs, and ranged from 8 to 35% in the 3 others. ACE activity was $75 \pm 9\%$ 2 days after this last dose, and returned to pretreatment value (ie, approximately 100%) 5 days later. Under renal-impaired conditions, after the 8th PO administration, ACE activity was abolished in all but 1 dog between 1 and 4 hours. ACE activity was $69 \pm 10\%$

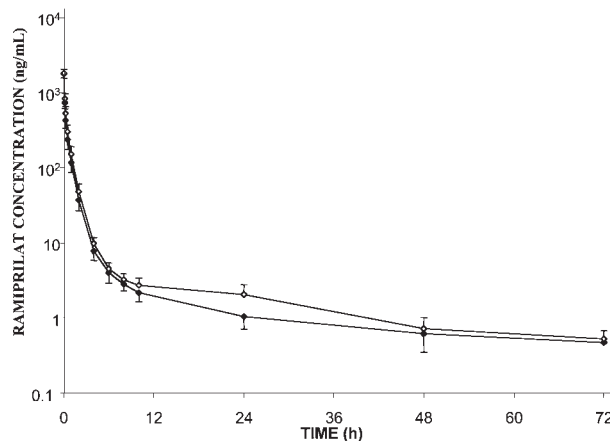


Fig 1. Mean \pm standard deviation plasma concentration of ramiprilat (ng/mL) versus time (in hours) profile, after an IV bolus administration of ramiprilat at a dosage of 0.25 mg/kg in 10 dogs under healthy (black circles) and renal-impaired (white circles) conditions.

and $93 \pm 7\%$ 2 and 5 days after this last dose, respectively.

There were statistically significant differences between healthy and renal-impaired conditions after IV bolus administration of ramiprilat for the clearance of the free fraction and IC_{50} , which were decreased by approximately 14 and 49%, respectively, under renal-impaired conditions. The half-life of elimination of the free fraction was significantly increased by 17%. However, after repeated PO administration of ramipril, no significant change was observed for any parameter.

Discussion

The model of renal impairment used here was 1st described in dogs in 1990 and has been used for assessing PK alterations of various drugs under renal-impaired conditions.^{11,15,18} This model allows the induction of various degrees of renal dysfunction depending on the extent of renal cortical electrocoagulation.¹⁵ Morphine

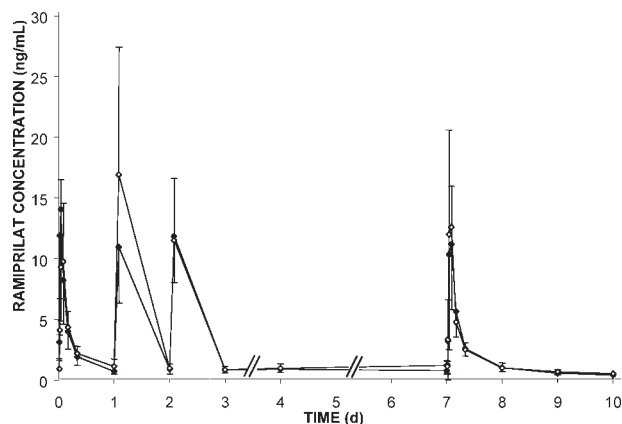


Fig 2. Mean \pm standard deviation plasma concentration of ramiprilat (ng/mL) versus time (in days) profile, after repeated PO administration of ramipril at a dosage of 0.25 mg/kg (once a day) for 8 days in 10 dogs under healthy (black circles) and renal-impaired (white circles) conditions.

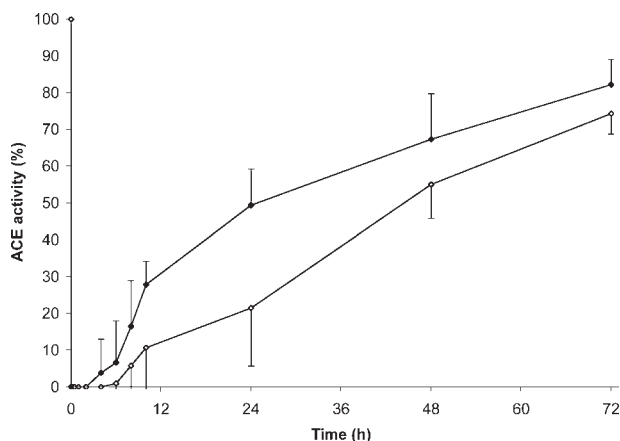


Fig 3. Angiotensin-converting enzyme (ACE) activity (% of the maximal value observed) versus time (in hours) profile, after IV bolus administration of ramiprilat at a dosage of 0.25 mg/kg in 10 dogs under healthy (black circles) and renal-impaired (white circles) conditions. Under renal-impaired conditions, the right shift of the curve is related to a decrease of the amount of ramiprilat required to produce 50% of the total inhibition of ACE.

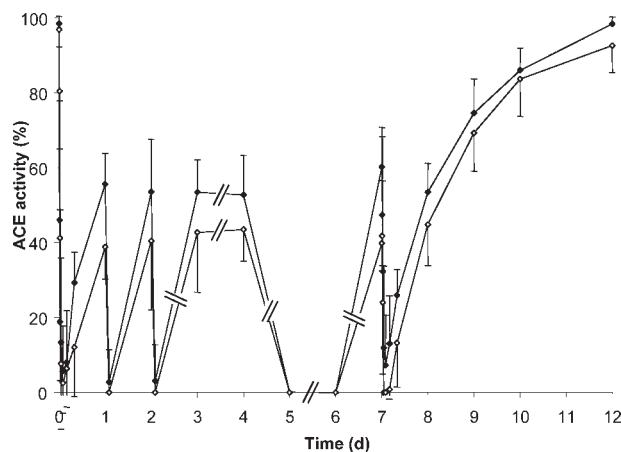


Fig 4. Angiotensin-converting enzyme activity (% of the maximal value observed) versus time (in days) profile, after repeated PO administration of ramipril at a dosage of 0.25 mg/kg (once a day) for 8 days in 10 dogs under healthy (black circles) and renal-impaired (white circles) conditions.

treatment provided adequate analgesia and allowed excellent recovery. On the day after surgery, the dogs were alert and regained their appetite. Control GFR values was similar to that previously described in healthy dogs.¹⁹ The decrease in GFR induced in the present study corresponds to moderate renal impairment. The dogs did not show any clinical signs during the postoperative period, except a slight decrease in body weight (approximately 3.5%). A 2-fold increase in plasma creatinine concentration was observed but most of the postoperative values remained below the upper

limit of the reference interval (ie, 1.47 mg/dL). Changes in plasma sodium, chloride, and bicarbonate concentrations were statistically significant but not clinically relevant.

Renal dysfunction was characterized by decreases in GFR and ERPF, estimated by plasma iohexol and PAH clearances, respectively, as described previously.¹⁶ GFR and ERPF results obtained under healthy conditions were similar to those published previously for Beagle dogs.¹⁶ Induction of renal impairment induced a decrease in GFR of approximately 60%, which corresponds to a moderate degree of renal dysfunction in comparison to

Table 2. Effect of renal impairment on the pharmacokinetic and pharmacodynamic parameters of ramiprilat in 10 adult Beagle dogs after a single bolus IV administration of ramiprilat (at a dosage of 0.25 mg/kg).

Variable		Condition	
		Healthy	Renal-Impaired
V_c	(L/kg)	0.11 ± 0.016	0.11 ± 0.013
Cl_{free}	(L/kg/h)	0.51 ± 0.089	0.43 ± 0.088**
$t_{1/2}K_{10}$	(min)	9.4 ± 1.7	11.0 ± 1.4**
B_{max}	(nM)	142 ± 30.4	142 ± 31.3
	(ng/L)	55,161 ± 11,809	55,161 ± 11,809
P_{max}	(nM)	7.6 ± 2.56	9.7 ± 2.51
	(ng/L)	2,952 ± 994	3,768 ± 975
K_d	(nM)	0.76 ± 0.455	0.90 ± 0.334
	(ng/L)	295 ± 177	350 ± 130
f_{circ}	(%)	5.6 ± 2.3	7.2 ± 2.5
IC_{50}	(nM)	0.399 ± 0.234	0.205 ± 0.064*
	(ng/L)	155 ± 91	80 ± 25*
n		0.997 ± 0.259	1.412 ± 0.488

V_c , volume of distribution of the free fraction (ie, free plus fraction not specifically bound to angiotensin-converting enzyme [ACE]); Cl_{free} , clearance of the free fraction; $t_{1/2}K_{10}$, half-life of elimination of the free fraction; B_{max} , total binding capacity (circulating and noncirculating ACE) scaled by V_c ; P_{max} , plasma binding capacity scaled by V_c ; K_d , concentration of free ramiprilat producing 50% saturation of converting enzyme (circulating and noncirculating); f_{circ} , fraction of total binding site (B_{max}) that is not located onto tissue; IC_{50} , plasma ramiprilat concentration required to produce 50% of the total inhibition; n, a power term representing the steepness of the concentration-effect curve. Molar mass of ramiprilat is 388.46 g/mol.

* $P < .05$; ** $P < .01$.

Table 3. Effect of renal impairment on the pharmacokinetic and pharmacodynamic parameters of ramiprilat in 10 adult Beagle dogs after repeated PO administration of ramipril (at a dosage of 0.25 mg/kg once a day for 8 days).

Variable		Condition	
		Healthy	Renal-Impaired
V_d/F	(L/kg)	2.16 ± 1.039	2.38 ± 0.914
Cl_{free}/F	(L/kg/h)	8.38 ± 3.338	7.34 ± 2.758
$t_{1/2}K_{10}$	(min)	11.5 ± 6.0	14.9 ± 7.2
B_{max}	(nM)	145 ± 64.4	138 ± 44.2
P_{max}	(ng/L)	56,327 ± 25,017	53,607 ± 17,170
P_{max}	(nM)	4.9 ± 1.90	5.7 ± 2.41
K_d	(ng/L)	1,903 ± 738	2,214 ± 936
K_d	(nM)	1.062 ± 0.665	1.219 ± 0.485
K_d	(ng/L)	413 ± 258	474 ± 188
f_{circ}	(%)	3.8 ± 2.1	7.4 ± 9.7
$AUC_{free, trap}$	(nmol/h/L)	698 ± 290	808 ± 355
$AUC_{free, trap}$	(ng/h/L)	271,145 ± 112,653	313,876 ± 137,903
F	(%)	6.7 ± 2.0	6.6 ± 3.4
IC_{50}	(nM)	0.646 ± 0.414	0.452 ± 0.250
IC_{50}	(ng/L)	251 ± 161	176 ± 97
N		0.844 ± 0.247	1.067 ± 0.212

V_d/F , volume of distribution of the free fraction (ie, free plus fraction not specifically bound to angiotensin-converting enzyme [ACE]); Cl_{free}/F , clearance of the free fraction; $t_{1/2}K_{10}$: half-life of elimination of the free fraction; B_{max} , total binding capacity (circulating and noncirculating ACE) scaled by V_d ; P_{max} , plasma binding capacity scaled by V_d ; K_d , concentration of free ramiprilat producing 50% saturation of ACE (circulating and noncirculating); f_{circ} , fraction of total binding site (B_{max}) that is not located onto tissue; $AUC_{free, trap}$, area under the free plasma concentration versus time curve determined by the trapezoidal rule; F, oral bioavailability of ramiprilat from ramipril; IC_{50} , plasma ramiprilat concentration required to produce 50% of the total inhibition; n, a power term representing the steepness of the concentration-effect curve. Molar mass of ramiprilat is 388.46 g/mol.

the 75% loss in renal function expected to cause clinical signs. The decrease in ERPF also was approximately 60%. Filtration fraction remained approximately the same (ie, 30%). In heart failure, as in the present model, both GFR and ERPF may be decreased, as shown in experimental canine models of heart failure.^{20,21} Moreover, if a decrease in renal function occurs in dogs with heart failure, it most often is subclinical, which means that the decrease in renal function induced in our study is representative of the degree of renal dysfunction expected in dogs with heart failure (ie, the target group for ramipril).

The potential effects of drug treatment on plasma biochemical variables are not only of relevance for identification of potential adverse effects, but also to allow adequate interpretation of changes in biochemical markers used for monitoring a disease condition (eg, serum creatinine concentration for renal failure). Treatment with ramipril for 8 days in healthy dogs caused a slight increase in plasma sodium and chloride ion concentrations. Under renal-impaired conditions, only a slight increase in the plasma bicarbonate concentration was observed. Under both conditions, these changes were negligible and are not considered clinically relevant electrolyte disturbances. Other plasma variables, including plasma potassium concentration, were unaltered. Nevertheless, these findings should be further evaluated by long-term follow-up studies in ramipril-treated dogs. The effects of other ACEIs on biochemical variables in dogs with heart failure have been investigated previously and mild alterations have been described. After 3 weeks of enalapril treatment, plasma

potassium concentrations increased slightly,²² and a decrease in serum phosphorus concentration was observed which was within the reference interval.²³ Most often, however, no changes in blood biochemical variables have been reported. Long-term treatment with quinapril or captopril did not affect blood urea, creatinine, sodium, and potassium concentrations.²⁴ Similar observations were made for plasma potassium, urea, and ALT concentrations during long-term benazepril treatment.²⁵ Serum creatinine concentration has been shown to increase slightly,²⁶ remain unchanged,²⁷ or decrease²⁵ during ACEI treatment.

The main objective of the present study was to determine PK and PD parameters of ramipril in healthy and renal-impaired dogs. PK/PD analysis was performed by using a modeling approach developed for the dog.¹² This approach was proposed because ACEI disposition cannot be appropriately explained by interpreting multiexponential equations in terms of conventional compartmental models. For example, a classical interpretation is unable to explain why there is no accumulation of ACEI during repeated administration despite a long terminal half-life or why the lag time to absorption was longer than the time to the 1st observable effect on ACE activity when the 1st blood sampling was performed within the 1st 15 minutes after ACEI dosing (see Toutain and Lefebvre¹⁷ for explanation). Adequate interpretation of plasma ACEI concentration profiles requires a physiologically based model that takes into account the saturable binding of ACEI to both serum and tissue ACE. The plasma ACEI concentration-time profile actually exhibits 2 phases,

an initial elimination phase that reflects clearance of free drug and a protracted 2nd phase that is controlled by the release of drug, mainly from tissue-binding sites, before elimination from the body.

Single IV bolus administration of ramiprilat was performed because it was the only way to determine the basic PK parameters of the active moiety. In healthy dogs, after an IV bolus of ramiprilat, clearance was estimated to be 0.51 L/kg/h. When using similar PK modeling, the plasma clearance of benazeprilat was 2.5-fold lower (0.22 L/kg/h).¹² The plasma clearance of ramiprilat is rather high and represents approximately 10% of cardiac output. The volume of distribution of ramiprilat refers only to V_c here because the physiologically based model consists of a single compartment. The value was smaller than that of benazeprilat (0.2 L/kg, which corresponds to extracellular fluid volume).¹² This observation is relevant to the PD effects because the ACEs are ectopeptidases anchored to the cell surface with the catalytic site exposed at the extracytoplasmic surface.²⁸ Interindividual variability was small and the coefficients of variation were 15 and 17%, respectively, for plasma clearance and volume of distribution. The elimination half-life of free ramiprilat ($t_{1/2}K_{10}$, equal to $0.693/K_{10}$) was 9.4 minutes (ie, approximately 5 times lower than the half-life of elimination of benazeprilat).¹² The very short half-life of ramipril is explained by both the smaller volume of distribution and higher clearance, because half-life is a hybrid parameter that depends on both clearance and volume of distribution. Drug accumulation during multiple dosing therefore is unlikely to occur, even in severe disease conditions, with the recommended once-a-day dosing schedule. Actually, the observed terminal half-life ($t_{1/2\lambda_z}$) is governed by the nonlinear saturable binding of ramiprilat to ACE. It is in fact the high affinity of ramiprilat for ACE, and not the elimination process, that explains the very long terminal half-life. For this monocompartmental model, it can be demonstrated that $t_{1/2\lambda_z}$ is equal to $t_{1/2}K_{10} \times B_{\max}/K_d$ ¹⁷ (ie, here approximately 28 hours). This value is close to the terminal half-life value (24 hours) determined by fitting of our data using a conventional compartmental approach (data not shown). The initial decreasing phase of the plasma concentration versus time profile reflects the elimination process (ie, clearance of free ramiprilat), and not the distribution phase. During this phase, ACE is saturated, explaining rapid drug elimination.

The oral PK study was performed using multiple dosing because commercially available ACEI are administered chronically to dogs with heart failure. The bioavailability of ramipril (~7%) after PO administration is quite low and of the same magnitude as that for benazepril (~4%) after multiple dosing.¹² These findings indicate that most of the prodrug administered PO is not absorbed or biotransformed by the liver to the active metabolite. Large interindividual variability in oral bioavailability (approximately 30 and 50% under healthy and renal-impaired conditions, respectively) is a consequence of these metabolic findings and may contribute to interindividual differences in drug expo-

sure after oral administration. These features probably do not explain alterations in ACE inhibition and response to the treatment because of the saturation of the ACE whatever the level of exposure.

Under healthy conditions, ACE activity was abolished immediately after a single IV bolus of ramiprilat and mean values were approximately 50 and 80%, respectively, of normal ACE activity 1 and 3 days later. During repeated administration PO, average ACE activity never exceeded 60% of basal activity. The physiologically based model allows determination of maximal ACE binding capacity (B_{\max}), which was estimated to be 142 nM. Approximately 95% of the ACE pool is not circulating. Similar values were found for multiple PO dosing. These results are quite close to those previously obtained using the same approach when studying the disposition of benazeprilat (B_{\max} 112–160 nM, f_{circ} 10%), assuming similarly a 1:1 binding relationship for ACEI and ACE. This observation is not surprising because B_{\max} , as well the fraction of ACE that is not bound to tissue, is a dog-related and not drug-related property. K_d (ie, the concentration of free drug producing saturation of 50% of the entire ACE pool) conversely is a drug-dependent parameter that measures the affinity of a drug for ACE. In comparison to the value obtained for ramiprilat (1.1 nM), K_d values for benazeprilat and enalaprilat, determined after PO dosing, were higher (4.2 and 7.4 nM, respectively),¹³ possibly indicating that ramiprilat has a greater affinity for ACE. The IC_{50} of ramiprilat (0.65 nM) is lower than that of enalaprilat (1.09 nM) but higher than that of benazeprilat (0.27 nM) determined previously under healthy conditions.¹³ The Hill coefficient of ramiprilat was similar to values calculated for benazeprilat and enalaprilat (0.60–1.09),¹³ indicating that the steepness of the concentration-effect curve was similar for all 3 ACEIs.

Under renal-impaired conditions, plasma clearance of free ramiprilat and IC_{50} after IV administration were decreased 14 and 49%, respectively. The decrease in plasma clearance, which explains the increase in half-life of elimination, was very mild and cannot be considered clinically relevant. This finding may be explained by the predominantly hepatic excretion. Urinary excretion of ramiprilat is indeed minor in dogs because it was shown that, after PO administration of ¹⁴C-ramipril, radioactivity recovered from urine amounted to only 15% of the administered dose.⁶ The decrease in IC_{50} was more pronounced, despite an unchanged K_d . In other words, the only apparently biologically relevant change in ramiprilat PD was IC_{50} , determined ex vivo. In fact, IC_{50} is a hybrid parameter, which depends not only on K_d (ie, the affinity of the drug for ACE), but also on τ (ie, the efficacy of an agonist or antagonist in a tissue), which here represents the efficiency of transduction of ramiprilat-bound ACE into inhibitory effect on ACE activity ($IC_{50} = K_d/(1 + \tau)$).²⁹ IC_{50} consequently is always smaller than K_d . The discrepancy in the effect of renal impairment on K_d and IC_{50} could be explained by changes in enzyme activity due to alterations in the plasma composition under renal-impaired conditions. Surprisingly, no effect of renal impairment was observed

on PK and PD parameters after multiple PO dosing. In a previous study in dogs with renal impairment, a mild decrease in plasma clearance of marbofloxacin was noticed without any impact on PK parameters after repeated PO administration.¹⁸ These examples indicate that mild alterations in PK parameters after IV bolus administration does not necessarily affect drug disposition after repeated PO administration. The K_d and IC_{50} of ramiprilat, after repeated PO administration of ramipril, were not affected by renal impairment. These results indicate clearly that the disposition of ramiprilat, administered according to the current recommendations, is not altered in dogs with a 60% decrease in GFR. In humans, the PK of ramiprilat are modified in patients with various degrees of renal impairment, because most of the drug is excreted in the urine as ramiprilat and its glucuronate conjugate.⁵ Interestingly, the bioavailability of ramiprilat, although showing considerable interindividual variability, remains unchanged whatever the renal status. No information is available about the effect of renal impairment on the bioavailability of enalaprilat and benazeprilat. Although gastrointestinal disturbances frequently are observed in dogs with chronic renal failure, the effect of renal dysfunction on the PO bioavailability of drugs in dogs rarely has been documented. Changes in intestinal motility, which may affect bioavailability, appear to occur early in the course of disease and were identified in dogs with subclinical chronic renal impairment.³⁰ In humans, however, absorption of ACEI from the gastrointestinal tract and conversion of these prodrugs to active compounds generally is considered not to be affected in patients with renal failure.³¹

In conclusion, the results obtained in the present study confirm data previously obtained during PK studies involving benazepril and enalapril, especially the value of B_{max} and the low bioavailability of ACEI. The current recommended dosage of ramipril in dogs allows adequate inhibition of ACE activity. Moreover, ramipril did not alter plasma biochemical variables in healthy and renal-impaired dogs and there is no need for dosage regimen adjustment of ramipril in dogs with moderate renal impairment.

Footnotes

^a Royal Canin M25, Aimargues, France

^b Calmivet, Vétquinol, Lure, France

^c Nesdonal, Merial, Lyon, France

^d Forene, Abbott, Rungis, France

^e Morphine Lavoisier, solution injectable 10 mg/mL, Chaix & Du Marais, Paris, France

^f Rilexine Poudre injectable, Virbac, Carros, France

^g Ektachem 700 XR, Kodak, Johnson and Johnson Clinical Diagnostic Europe, Illkirch Graffenstaden, France

^h PAH, Sigma, Saint Quentin Fallavier, France

ⁱ Omnipaque 300, Nycomed Imaging AS, Oslo, Norway

^j Ramiprilat solution, Intervet Pharma R&D, Beaucouzé, France

^k Vasotop 1.25 and Vasotop 2.5, Intervet Pharma R&D, Beaucouzé, France

^l Alliance 2690, Waters, Milford, MA

^m Quattro Liquid Chromatograph, Micromass, Manchester, UK

ⁿ STRATA SDB-L cartridges, AiT France, Le Mesnille Roi, France

^o Radioenzymatic assay kit (ACE Direct_REA_kit), Bühlmann Laboratories AG, Allschwil, Switzerland

^p WinNonLin, Version 4.0.1, Pharsight, Mountain View, CA

^q Systat Version 8.0, SPSS Inc., Chicago, IL

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