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# Effect of amlodipine on the circulating renin-angiotensin-aldosterone system in healthy cats

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## Abstract

**Background:** Systemic hypertension (SH) is a common cardiovascular disease in older cats that is treated primarily with the calcium channel blocker amlodipine besylate (AML). The systemic effect of AML on the classical and alterative arms of the renin-angiotensin-aldosterone system (RAAS) in cats is incompletely characterized.

**Hypothesis/Objectives:** To determine the effect of AML compared to placebo on circulating RAAS biomarkers in healthy cats using RAAS fingerprinting.

**Animals:** Twenty healthy client-owned cats.

**Methods:** Cats were administered amlodipine besylate (0.625 mg in toto) or placebo by mouth once daily for 14 days in a crossover design with a 4-week washout period. Plasma AML concentrations and RAAS biomarker concentrations were measured at multiple timepoints after the final dose in each treatment period. Time-weighted averages for RAAS biomarkers over 24 hours after dosing were compared between treatment groups using Wilcoxon rank-sum testing.

**Results:** Compared to placebo, AML treatment was associated with increases in markers of plasma renin concentration (median 44% increase; interquartile range [IQR] 19%-86%; $P = .009$), angiotensin I (59% increase; IQR 27-101%; $P = .006$), angiotensin II (56% increase; IQR 5-70%; $P = .023$), angiotensin IV (42% increase; −19% to 89%; $P = .013$); and angiotensin 1-7 (38% increase; IQR 9-118%; $P = .015$).

**Conclusions and Clinical Importance:** In healthy cats, administration of AML resulted in nonspecific activation of both classical and alternative RAAS pathways.

KEYWORDS
angiotensin-converting enzyme inhibitor, blood pressure, cardiovascular agents (pharmacology), feline (species), systemic hypertension, vasodilator

**Abbreviations:** AA2, marker of adrenal responsiveness to angiotensin II (ratio of aldosterone to angiotensin II); ACE, angiotensin-converting enzyme; ACE-S, marker of angiotensin-converting enzyme activity (ratio of angiotensin II to angiotensin I); ALD, aldosterone; AML, amlodipine besylate; Ang, angiotensin; AUC, area under the curve; BP, blood pressure; IQR, interquartile range; LLOQ, lower limit of quantification; PK, pharmacokinetic; PRA-S, marker of plasma renin concentration (sum of angiotensin I and angiotensin II); RAAS, renin-angiotensin-aldosterone-system; SH, systemic hypertension; TWA, time-weighted average.

## 1 | INTRODUCTION

Systemic hypertension (SH), or sustained high blood pressure (BP), is a common cardiovascular disorder affecting older cats,[1] occurring most commonly secondary to chronic kidney disease[2,3] or hyperthyroidism.[4,5] Untreated SH can lead to target organ damage such as retinal detachment, myocardial hypertrophy, hypertensive encephalopathy and glomerulosclerosis.[1] First-line medical therapy for SH in cats is amlodipine besylate (AML),[1] a dihydropyridine calcium-channel blocking agent that results in relaxation of vascular smooth muscle and subsequent arterial vasodilation.[6] Doses of 0.625-1.25 mg AML per cat once daily effectively lower BP[6,7] and decrease proteinuria[8] in cats with SH. Despite the clinical efficacy of amlodipine, questions remain regarding the potential effects of AML on the activation of the renin-angiotensin-aldosterone system (RAAS).

In dogs, AML activates the classical RAAS,[9,10] presumably due to its BP-lowering effect. Based on this information, ACVIM consensus guidelines for management of SH[1] currently recommend against monotherapy with AML for dogs with SH, instead favoring combination therapy with a RAAS inhibiting medication such as an ACE inhibitor. In cats with SH, treatment with AML is associated with increases in plasma renin activity with no change in ALD.[11] However, previous observations about the effect of AML on the RAAS are largely based on elevations in serum or urine ALD concentrations, which are suboptimal endpoints to characterize RAAS activity[12-14] and do not consider the alternative RAAS pathway. A comprehensive RAAS fingerprinting assay reveals activation of both classical and alternative RAAS pathways in hypertensive cats treated with AML compared to healthy cats or hypertensive cats not receiving AML.[15] No published studies have compared comprehensive RAAS activity in healthy cats before and after AML treatment in a prospective placebo-controlled setting.

The effect of AML on the classical and alternative RAAS pathways in cats therefore remains incompletely characterized. The objective of the present study was to evaluate the pharmacodynamic response of AML administration (compared to placebo) on a comprehensive array of circulating RAAS components from both classical and alternative pathways in healthy cats. We hypothesized that AML treatment would result in higher concentrations of all RAAS peptides compared to placebo.

## 2 | MATERIALS AND METHODS

### 2.1 | Animals

Results of an a priori sample size calculation indicated that 20 cats (2 groups of 10 cats) would be required to detect a mean difference of 250 pmol/L in AngII levels between groups with 90% power and an $\alpha$ of .05. This calculation was chosen based on the mean difference in AngII levels noted between AML-treated cats with SH and untreated cats in a previous study, utilizing the SD in AngII levels from either healthy cats or cats with SH (both ~200 pmol/L).[15] The study protocol was approved by the Iowa State University Institutional Animal Care and Use Committee (IACUC no. 21-025). Cats were owned by students or staff of the Iowa State University College of Veterinary Medicine and owner consent was obtained for each cat prior to study enrollment. While use of experimental cats was considered for this study, ultimately the authors determined that use of client-owned cats better supported cat well-being by allowing typical at-home housing and enrichment throughout the study, ensuring voluntary participation and informed consent by caregivers, and avoiding the need to purchase and subsequently re-home purpose-bred cats. Safety profiles of study medications were thoroughly discussed with owners prior to enrollment, and all owners accepted that the benefits of a comprehensive medical workup for their personal cat and long-term scientific benefit of the study outweighed the risk of short-term discomfort of diagnostic testing during study visits.

All cats were older than 5 years, presumed healthy, and receiving no oral medications other than routine preventatives. The health status of each of cat was determined at the first study visit by physical examination, BP, complete blood count and serum chemistry panel, total thyroxine level (for cats greater than 8 years of age), and echocardiography performed by a board-certified cardiologist. Systolic BP was estimated from the coccygeal artery with a Doppler device using standardized methods.[1] Cats were excluded from the study if health screening identified systemic or cardiac disease or BP >160 mm Hg. Cats that were too fractious to perform routine physical examination, venipuncture, and echocardiography without sedation were also excluded. Cats were required to consume a consistent diet within each cat (controlling for sodium consumption) and to be meal-fed at consistent feeding times throughout the study.

### 2.2 | Study design and medications

The study was a prospective double-blinded trial with a 58-day observation period, consisting of 3 study visits: Visit 1 (Day 0), Visit 2 (Days 14-15), and Visit 3 (Days 57-58; see Figure 1). This visit schedule was designed such that Visits 2 and 3 would capture pharmacokinetic (PK) steady state of AML (>5 half-lives; half-life of AML in cats previously estimated at 53 hours[16]) while allowing adequate washout (>10 half-lives) between treatments.

At Visit 1, baseline measurements (physical examination, BP, echocardiography, complete blood count, serum chemistry, total thyroxine level [for cats greater than 8 years of age], and comprehensive RAAS fingerprint) were obtained for all cats in the morning (0 hour, 08:00; see Figure 1). After study inclusion, cats were randomized by sex to receive either AML 0.625 mg PO once daily (Group A) or identical placebo PO once daily (Group B) for a period of 14 days (Days 1-14). Cats then underwent a 4-week washout period before receiving the alternative treatment for 14 days (Days 45-57). All medication doses were administered by owners at home at approximately 08:00 with food, except the final dose for each treatment period which was administered by study personnel on the morning of Visits 2 and

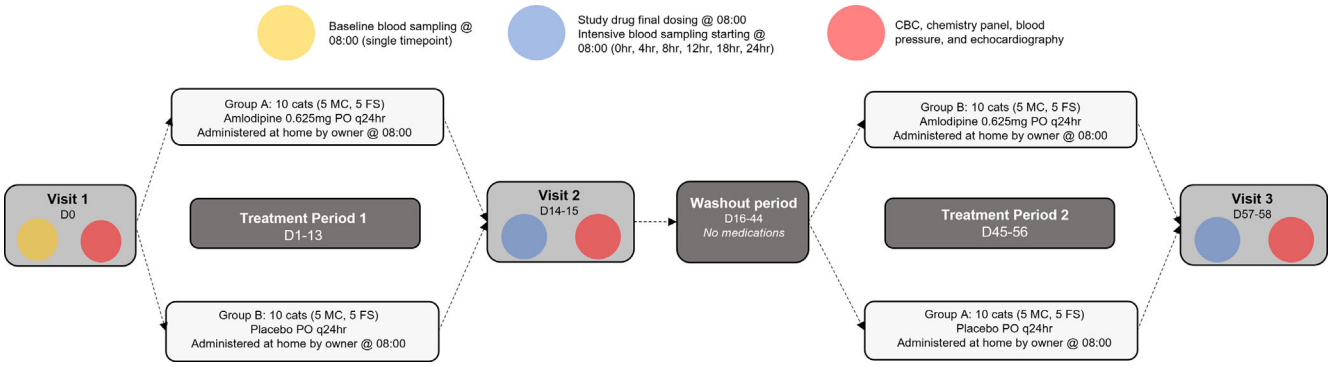


**FIGURE 1** Schematic representation of crossover study design and timeline for sample collection. There are 2 treatment periods during which randomized groups of 10 cats cross over to receive either amlodipine besylate (0.625 mg) or identical placebo capsule PO once daily (q24hr). Between treatment periods, there is a 4-week washout period. Diagnostic testing performed at each timepoint is indicated by color-coded shaded circles. See text for further details. D, day of the study period.

3 (Day 14 and 57). On the morning of Visits 2 and 3 (on Days 14 and 57), owners also administered gabapentin 50 mg by mouth 30 minutes before the study visit (at approximately 07:30) to decrease anxiety associated with hospitalization and repeated venipuncture.

Study medications were compounded by the Iowa State University Lloyd Veterinary Medical Center Pharmacy (active: amlodipine pure USP powder at 0.625 mg strength with lactose powder; placebo: lactose powder) and packaged into gelatin capsules of identical size but different colors. Cat owners and all personnel except for the study coordinator (TGM) were blinded to treatment group. Active capsules were tested for potency on the day of compounding and at the conclusion of the study to confirm >90% potency of AML.

For Visit 2 (Days 14-15) and Visit 3 (Days 57-58), cats returned to the study site for overnight monitoring and intensive blood sampling at 0, 4, 8, 12, 18, and 24 hours (08:00, 12:00, 16:00, and 20:00 on Days 14 and 57; 02:00 and 08:00 hour on Days 15 and 58; see Figure 1). After blood sampling at 0 hour, cats were fed (diet provided by owners) and the final dose of each study treatment (AML or placebo) was administered PO. Blood samples were obtained for quantification of RAAS biomarkers (all cats, all timepoints); plasma AML concentrations (AML groups only, all timepoints; blood aliquoted by non-blinded study coordinator); and CBC and serum chemistry (all cats, 24 hour timepoint only, to assess for any adverse biochemical effects of study treatments). Cats were again fed the diet provided by owners at 12 and 24 hours. On the final blood collection on the second day of each intensive sampling period (Days 15 and 58), BP and echocardiography were repeated to assess hemodynamic effects of study treatments. The intensive PK/PD sampling schedule was chosen to optimize the opportunity for subsequent mathematical modeling of the exposure-response relationship of AML on the RAAS. Such models, which are forthcoming from our research group, can then be refined and enriched using sparse clinical sampling of cats with naturally-occurring systemic hypertension to simulate "virtual clinical trials" of AML in diseased cats.

## 2.3 | Data collection

Peripheral venous blood samples were collected from external jugular, medial saphenous, or cephalic veins by use of 22- or 25-gauge needles or butterfly catheters attached to 3- to 6-mL syringes. From each cat, 6 mL of whole blood was obtained at Visit 1 (single timepoint at 0 hour), and a total of 16 mL of whole blood was obtained at Visits 2 and 3 (3 mL at 0 hour; 2 mL at 4, 8, 12, and 18 hours; 5 mL at 24 hours). Complete blood counts and serum chemistry panels were performed at the Iowa State University Lloyd Veterinary Medical Center Clinical Pathology Laboratory.

Samples to be used for quantification of RAAS biomarkers were collected into additive-free tubes and centrifuged at 1500×*g* for 15 minutes to obtain 0.5 mL of serum per timepoint, which was frozen at −80°C for batch analysis. For AML PK, whole blood was collected into chilled EDTA tubes and centrifuged at 1500×*g* and 4°C for 15 minutes, with supernatant frozen at −80°C for batch analysis.

## 2.4 | Plasma amlodipine quantification

Pharmacokinetic analysis of AML metabolites was performed at the Iowa State University Analytical Chemistry Laboratory using liquid chromatography-mass spectrometry as described below. The amlodipine analytical standard was commercially purchased (Cayman Chemical, Ann Arbor, MI, USA). A stock standard solution was made at 1 mg/mL in DMSO. Spiking solutions were prepared in acetonitrile. Amlodipine-d4 (Cayman Chemical, Ann Arbor, MI, USA) was used as the internal standard.

A total sample volume of 200 µL was used for the extraction. A protein precipitation was performed by adding 800 µL of acetonitrile +0.5% formic acid to each sample. Samples were vortexed by hand for approximately 30 seconds and centrifuged at 10 600×*g* for 5 minutes. Eight hundred microliters of supernatant was transferred to a glass tube and evaporated to dryness under a nitrogen stream. All samples were reconstituted into 100 µL of 50:50 acetonitrile:

water. The injection volume on the analytical column was 2 µL. The linear range used for the analysis was 0.5-200 ppb, and the lower limit of quantification (LLOQ) was 0.5 ppb.

A Vanquish Flex liquid chromatography pump interfaced with a TSQ Altis mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) were used for the analysis. The source conditions were as follows: spray voltage 3500 V (positive polarity), sheath gas 20 Arb (arbitrary units), auxiliary gas 15 Arb, sweep gas 1 Arb, ion transfer tube temperature 325°C, and vaporizer temperature 300°C. The total run time of the method was 3 minutes. The resolution of Q1 and Q3 was 0.7 full width at half maximum. The collision-induced dissociation was set to 2 mTorr. The chromatographic peak width was 2 seconds and the cycle time was 0.1 second. Analytes were separated using an Accucore C18 50 × 2.1, 2.6 µm (Thermo Fisher Scientific, San Jose, CA, USA) analytical column. The mobile phases used for the analysis were the following: Mobile Phase A was water +0.1% formic acid and Mobile Phase B was acetonitrile +0.1% formic acid. The column oven temperature was set to 40°C. The flow rate of the method was 0.4 mL/minute. Chromatographic conditions were as follows: start at 2% B and hold for 0.5 minute, linear ramp to 100% B for 1.5 minute, hold at 100% B for 0.3 minute, drop to 2% B in 0.01 minute, and hold at 2% B for 0.69 minute.

## 2.5 | RAAS biomarker quantification

Equilibrium concentrations of angiotensin peptides and ALD were quantified in serum samples by liquid chromatography-mass spectrometry/mass spectroscopy performed at a commercial laboratory (Attoquant Diagnostics, Vienna, Austria), using previously validated and described methods,[17-19] after ex vivo equilibration for 1 hour at 37°C. Briefly, samples were spiked with a stable isotope-labeled internal standard for each angiotensin and a deuterated internal standard for ALD (aldosterone D4) after equilibration, and analytes were extracted using C18-based solid-phase extraction. Extracted samples were analyzed using mass spectrometry analysis with a reversed-analytical column (Acquity UPLC C18, Waters, Milford, MA, USA) operating in line with a quadrupole mass spectrometer (XEVO TQ-S, Waters, Milford, MA, USA) in multiple reaction monitoring mode. Internal standards were used to correct for analyte recovery across the sample preparation procedure in each individual sample. Analyte concentrations were calculated from integrated chromatograms considering the corresponding response factors determined in appropriate calibration curves in serum matrix, when integrated signals exceeded a signal-to-noise ratio of 10. The lower limits of quantification (LLOQ) for the analytes in feline serum were 3 pmol/L (AngI), 2 pmol/L (AngII), 2.5 pmol/L (AngIII), 2 pmol/L (AngIV), 2.5 pmol/L (Ang1-7), 2 pmol/L (Ang1-5), and 13.9 pmol/L (ALD), respectively. Angiotensin-based markers for renin (PRA-S) and angiotensin converting enzyme (ACE-S) were derived from AngII and AngI concentrations by calculating their sum and ratio, respectively, whereas the ratio of ALD/AngII (AA2) was calculated to assess adrenal responsiveness after AngII signaling resulting in the release of ALD as described by the analytical laboratory.[19-21]

## 2.6 | Statistical analysis

Statistical analyses were performed using commercial software (R software, version 3.5.1, R Foundation for Statistical Computing, Vienna, Austria; IBM SPSS Statistics 25.0, IBM Corporation, Armonk, NY, USA). Normality of data was determined using the Shapiro-Wilk test. Quantitative data were summarized as mean ± SD for normally distributed data, and as median (interquartile range) for non-normally distributed data.

Pharmacokinetic analysis of amlodipine total plasma concentrations was performed using a statistical moment (ie, non-compartmental) approach in commercial software (PKanalix, MonolixSuite 2023R1, Lixoft, France). Standard PK parameters were generated for individual cats as follow:

- Maximum amlodipine plasma concentration at steady state, $C_{max}$;
- Time of maximum amlodipine plasma concentration at steady state, $T_{max}$;
- Area under amlodipine plasma concentration-time curve for the dosing interval *tau* (24 hours), $AUC_{tau}$;
- Average amlodipine plasma concentration at steady state, $C_{avg} = AUC_{tau}/24$;
- Slope of the terminal phase, computed by linear regression of the logarithmic amlodipine concentration vs time curve during the elimination phase, $\lambda_z$;
- Amlodipine plasma terminal half-life, $T_{1/2(\lambda z)} = \ln(2)/\lambda_z$;
- Amlodipine apparent systemic clearance at steady state, $CL_{ss}/F = Dose/AUC_{tau}$;
- Apparent volume of distribution of amlodipine during the elimination phase, $V_z/F = Dose/(AUC_{tau} \times \lambda_z)$.

For data analysis, the first amlodipine concentration after dosing below the LLOQ was inferred to be LLOQ/2, and subsequent data points were excluded from the analysis. A linear/log trapezoidal rule was used to estimate the area under amlodipine time curves. The slope of the terminal phase $\lambda_z$ was derived by linear regression between *Y* (log[concentrations]) and the *X* (time) using a $1/Y^2$ weighting method. Summary statistics on the individual PK parameters were performed thereafter to derive the Q1/Q2 (median)/Q3 quartiles, (min-max) range, geometric mean, and harmonic mean when appropriate (ie, for time-derived parameters).

Amlodipine $AUC_{tau}$ was compared between study groups (A vs B) using Wilcoxon rank-sum testing to assess for differences in AML exposure between randomized study groups. To assess any potential carryover effect on RAAS biomarkers between treatment periods, an analysis of variance was performed on the sum of time-weighted averages (TWA) for each biomarker using Benjamini-Hochberg correction and comparing to the null hypothesis (no carryover).

The effect of AML vs placebo on the classical and alternative arm of the RAAS was characterized by the area under the effect curve of each RAAS biomarker during the 24 hours after dosing. Calculation of the 24-hour TWA for each biomarker of interest (AngI, AngII, Ang III, AngIV, Ang1-7, Ang1-5, ALD, PRA-S, ACE-S, and AA2) was performed by dividing the area under the effect curve at steady state (determined using the trapezoidal rule) by the observation period (24 hours). Time weighted averages were compared between AML vs placebo groups using Wilcoxon rank-sum tests, and the extent of the effect was assessed by calculating the percent difference in TWA between AML vs placebo, as previously described.[22,23]

To evaluate the strength and direction of the monotonic relationship between $C_{avg}$ of AML and TWA of RAAS biomarkers, the Spearman's rank correlation method was used. Strength of correlations was classified as negligible (*r*-value of 0-.09), weak (.1-.39), moderate (.4-.69), strong (.7-.89), or very strong (.9-1).[24,25] Wilcoxon signed rank tests were used to compare BP results between treatment groups. For all analyses, *P*-values <.05 were considered as statistically significant.

## 3 | RESULTS

Twenty healthy client-owned cats were enrolled in this study, including 10 castrated males and 10 spayed females. Breeds represented included 14 domestic shorthair cats, 4 domestic longhair cats, and 2 Siamese. Median age of the cats was 8 years (range, 5-14), and median weight was 5.0 kg (range, 3.6-7.9 kg). None of the cats had known underlying systemic disease and none had systemic or cardiovascular disease identified during baseline diagnostic testing. No clinically relevant abnormalities in clinicopathologic variables (CBC and chemistry), BP, or echocardiographic variables were noted at any study visit in either treatment group.

Aggregated AML plasma concentration time-courses from both study periods are presented in Figure 2. Summary statistics of plasma PK parameters for AML are presented in Table 1. The median $AUC_{tau}$ for AML systemic exposure for 24 hours after dosing was 473.4 (interquartile range [IQR], 385.9-559.6) μg/L·h for group A and 487.7 (IQR 421.2-596.4) μg/L·h for group B, with no difference between

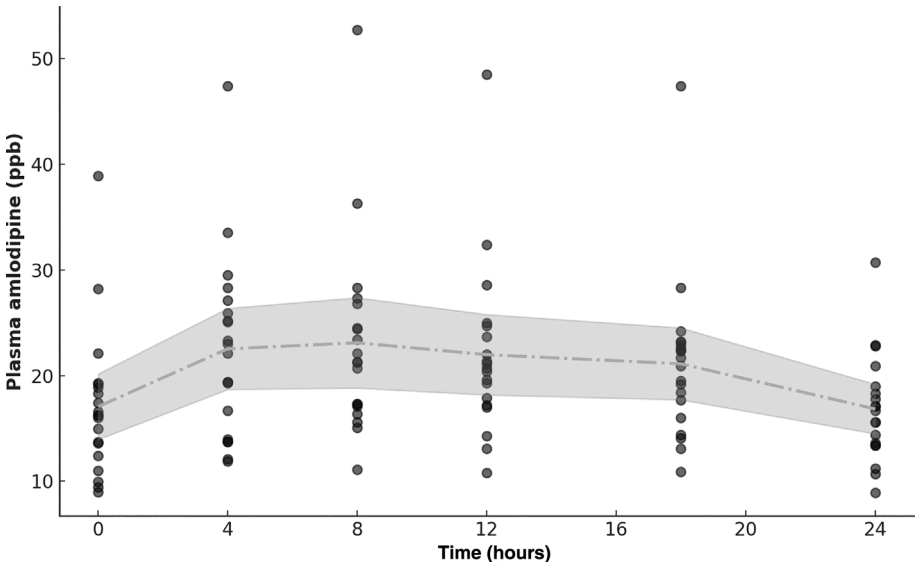


**FIGURE 2** Pharmacokinetic time-course of plasma amlodipine concentration in 20 healthy cats after the final dose of amlodipine administered at a dose of 0.625 mg PO q24hr for 14 days. The dotted gray line indicates mean plasma amlodipine concentration, the shaded gray area indicates 90% confidence interval of the mean, and circles indicate individual cat results. This figure was produced using Python, utilizing the *Matplotlib* library for data visualization (Python Language Reference version 3.8, Python Software Foundation, Wilmington, DE; available at: https://www.python.org).

**TABLE 1** Summary statistics of the plasma pharmacokinetic parameters for amlodipine after 14 days of treatment with 0.625 mg amlodipine besylate by mouth once daily in 20 healthy cats.

| Variable (unit) | Min | Q1 | Median | Q3 | Max | CV | Geo Mean | Geo CV | Harm mean |
|---|---|---|---|---|---|---|---|---|---|
| $C_{avg}$ (μg·L$^{-1}$) | 10.7 | 16.8 | 20.1 | 23.5 | 42.2 | 33.3 | 20.0 | 31.8 | |
| $C_{max}$ (μg·L$^{-1}$) | 12.1 | 20.1 | 23.9 | 27.8 | 52.7 | 35.1 | 23.5 | 33.0 | |
| $T_{max}$ (h) | 4.0 | 4.0 | 8.0 | 10.0 | 18.0 | 56.5 | 7.4 | 58.2 | 6.5 |
| $AUC_{tau}$ (μg·L$^{-1}$·h) | 256.7 | 403.6 | 483.2 | 565.1 | 1013.3 | 33.3 | 479.8 | 31.8 | |
| $\lambda_z$ (h$^{-1}$) | 0.0041 | 0.013 | 0.019 | 0.026 | 0.15 | 115.4 | 0.019 | 74.9 | 0.016 |
| $T_{1/2}$ ($\lambda_z$) (h) | 4.8 | 26.8 | 36.9 | 52.2 | 168.3 | 74.3 | 36.1 | 74.9 | 27.4 |
| $CL_{ss}/F$ (L·h$^{-1}$) | 0.6 | 1.1 | 1.3 | 1.5 | 2.4 | 30.9 | 1.3 | 31.8 | |
| $V_z/F$ (L) | 4.2 | 52.4 | 74.2 | 99.2 | 199.9 | 59.5 | 67.8 | 95.0 | |

*Note*: All data were generated using a statistical moment (ie, non-compartmental) approach in commercial software (PKanalix, MonolixSuite 2023R1, Lixoft, France). See text for complete definitions of pharmacokinetic variables.

Abbreviations: CV, coefficient of variation (%); GeoCV, geometric coefficient of variation; GeoMean, geometric mean; HarmMean, harmonic mean; Min, minimum; Q, quartile.

| Variable | Placebo | Amlodipine | Extent of treatment effect | *P*-value |
|---|---|---|---|---|
| AngI | 53.6 (38.6-64.0) | 87.9 (69.8-104.9) | 0.59 (0.27-1.01) | **.006** |
| AngII | 150.1 (88.1-180.9) | 278.7 (221.5-317.2) | 0.56 (0.04-0.70) | **.023** |
| ALD | 167.1 (123.5-211.8) | 197.2 (127.6-236.5) | 0.13 (−0.21-0.39) | .53 |
| Ang1-7 | 22.9 (12.9-34.1) | 41.0 (29.0-61.1) | 0.38 (0.09-1.18) | **.015** |
| Ang1-5 | 118.2 (66.2-167.0) | 159.0 (92.2-255.0) | 0.24 (−0.11-1.37) | .10 |
| AngIII | 8.1 (5.9-11.0) | 11.5 (6.5-16.7) | 0.41 (−0.17-0.56) | .23 |
| AngIV | 4.4 (3.2-6.3) | 7.6 (5.6-10.4) | 0.42 (0.19-0.89) | **.013** |
| PRA-S | 226.2 (149.1-277.4) | 384.4 (317.0-554.2) | 0.44 (0.19-0.86) | **.009** |
| ACE-S | 3.3 (2.8-4.1) | 3.5 (2.7-4.3) | 0.06 (−0.26-0.30) | .77 |
| AA2 ratio | 1.11 (0.72-1.62) | 0.69 (0.45-0.95) | −0.34 (−0.62-0.13) | **.021** |

**TABLE 2** Twenty-four hour time-weighted averages and extent of treatment effect of RAAS biomarkers in 20 healthy cats treated with 14 days of either amlodipine besylate (0.625 mg PO q24hr) or placebo in crossover design.

*Note*: Units for time-weighted averages of biomarkers is pmol/L; ratios are unitless. Data are presented as median (interquartile range). Extent of treatment effect is calculated as: (TWA for amlodipine − TWA for placebo)/(TWA for placebo). A positive treatment effect (eg, 0.59) indicates that values of AngI were 59% higher in the amlodipine group compared to placebo. A negative treatment effect (eg, −0.04) indicates that the values of that variable were 4% lower in the amlodipine group compared to placebo. Significant differences ($P < .05$) are indicated in bold.
Abbreviations: AA2, aldosterone to angiotensin II ratio (marker of adrenal responsiveness to angiotensin II); ACE-S, marker of angiotensin-converting enzyme activity; ALD, aldosterone; Ang, angiotensin; PRA-S, marker of plasma renin concentration.

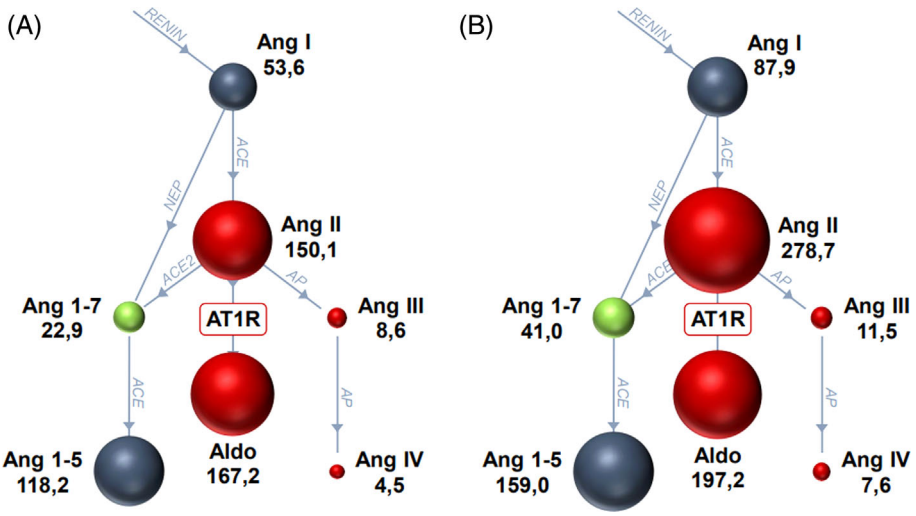


**FIGURE 3** Median values of 24-hour time-weighted averages of renin-angiotensin-aldosterone system biomarkers in 20 healthy cats treated with 14 days of either (A) placebo or (B) amlodipine besylate (0.625 mg PO q24hr) in crossover design. Sizes of circles are proportional to the median time-weighted average of each analyte. Aldo, aldosterone; Ang, angiotensin; AT1R, angiotensin type 1 receptor.

groups ($P = .68$). There was no evidence of a carryover effect between dosing groups for any RAAS biomarker tested ($P > .05$ for all study variables).

Comparisons of 24-hour TWA of RAAS biomarkers between AML-treated and placebo-treated cats are displayed Table 2 and Figure 3. Treatment effect size (percent difference between AML-treated and placebo-treated cats) is shown in Table 2. Overall, TWAs of PRA-S, AngI, AngII, Ang1-7, and AngIV were significantly higher in AML-treated cats compared to placebo, while TWA for the calculated AA2 ratio was lower in AML-treated cats compared to placebo.

Relationships between drug exposure and RAAS biomarkers in AML-treated cats is shown in Table 3. Overall, correlations between AML concentrations and RAAS biomarkers were not statistically significant.

Median BP before treatment was 127 mm Hg (interquartile range [IQR], 120-150 mm Hg). Median BP after placebo treatment was 131 mm Hg (IQR 119-136 mm Hg) and after AML treatment was 129 mm Hg (IQR 113-34 mm Hg). There was no significant difference between treatment groups in BP after treatment ($P = .51$), absolute change from baseline BP ($P = .51$), or percent change from baseline BP ($P = .55$).

## 4 | DISCUSSION

This study assesses the effect of AML on a comprehensive array of RAAS biomarkers in healthy cats. Overall, our results demonstrated that concentrations of RAAS biomarkers from both the classical and alternative pathways were higher in AML-treated cats compared to

**TABLE 3** Correlations (*r*-values and associated *P*-values) between 24-hour average amlodipine plasma concentration at steady state ($C_{avg}$) and 24-hour time-weighted average for RAAS biomarkers in 20 healthy cats treated with 14 days of amlodipine besylate (0.625 mg PO q24hr).

| Variable | Correlation coefficient (*r*-value) | *P*-value |
|---|---|---|
| AngI | 0.38 | .096 |
| AngII | 0.29 | .21 |
| ALD | −0.17 | .48 |
| Ang1-7 | 0.31 | .18 |
| Ang1-5 | 0.30 | .20 |
| AngIII | 0.28 | .23 |
| AngIV | 0.305 | .19 |
| PRA-S | 0.32 | .17 |
| ACE-S | −0.22 | .36 |
| AA2 ratio | −0.30 | .20 |

Abbreviations: AA2, aldosterone to angiotensin II ratio (marker of adrenal responsiveness to angiotensin II); ACE-S, marker of angiotensin-converting enzyme activity; ALD, aldosterone; Ang, angiotensin; PRA-S, marker of plasma renin concentration.

placebo. Specifically, AML-treated cats had higher concentrations of PRA-S (+44%), AngI (+59%), AngII (+56%), AngIV (+42%), and Ang1-7 (+38%) compared to placebo-treated cats. The increase in AngII also resulted in a lower AA2 ratio (34% decrease) in AML-treated cats.

These findings supported our hypothesis and are consistent with the existing literature reporting the effect of AML on the RAAS in cats. In 1 previous study of cats with SH, treatment with AML led to increased plasma renin activity with no change in ALD.[11] In another study utilizing a comprehensive RAAS fingerprint approach, hypertensive cats treated with AML showed increases in multiple biomarkers of both classical and alternative RAAS pathway (PRA-S, AngI, AngII, ALD, Ang1-7, AngIII, and AngIV) compared to healthy cats, and increases in PRA-S, AngI, and Ang1-7 compared to hypertensive cats not receiving AML.[15] Another study of AML-treated cats with SH similarly found higher concentrations of angiotensin peptides from both classical and alternative pathways.[26]

The finding that AML leads to nonspecific activation of both classical and alternative RAAS pathways is logical given its mechanism of action. As a vascular-specific calcium-channel blocker, AML vasodilates peripheral arterioles including the glomerular afferent arteriole. This decrease in BP is sensed both centrally via carotid baroreceptors and locally in the kidney and leads to juxtaglomerular release of renin, which metabolizes the liver-derived protein angiotensinogen to AngI, the precursor to both classical and alternative RAAS pathways. In dogs, AML activates the classical RAAS, and is used as an experimental model of RAAS activation in this species.[9,10] However, the effect of AML on the alternative RAAS pathway in dogs has not been investigated. Consensus guidelines for management of SH[1] recommend combining AML with a RAAS-inhibiting antihypertensive medication, such as an ACE or angiotensin-receptor blocker, due to this concern for classical RAAS activation. The same consensus guidelines do not currently include the same recommendation for cats; this may be partly due to paucity of studies investigating the effect of AML on the feline RAAS, as well as the high efficacy of AML as a monotherapy for treatment of hypertension in cats and lack of compliance with polypharmacy in this species.

The global effect of a drug on the RAAS is complex and depends on the balance between classical and alternative RAAS activation, as these 2 pathways have counterregulatory effects on fluid balance and vascular tone. Whether the precursor AngI is converted to AngII or Ang1-7 depends on the relative concentrations of the enzymes ACE, ACE2, and neprilysin. It is challenging to know what relative degree of classical vs alternative RAAS activation is clinically relevant. Our data demonstrate a 56% increase in AngII (classical pathway) and 38% increase in Ang1-7 (alternative pathway) after AML treatment in healthy cats; however, it remains unclear whether these differences would result in clinically meaningful outcome disparities in either healthy or diseased cats. Furthermore, the overall biologic effect of RAAS activation is determined not by circulating concentrations of angiotensins and ALD, but the interaction of these molecules with target receptors. Although classical RAAS activation could be characterized as an adverse effect of AML treatment, concurrent increased activity of the alternative RAAS pathway might lessen this effect or even contribute to the therapeutic benefit of AML. Specifically, increased activity of Ang1-7 may provide cardioprotective long-term benefits through vasodilatory, natriuretic, antihypertensive, anti-inflammatory and antifibrotic mechanisms.[27] Ultimately, our data do not provide a clear answer to the question of whether the RAAS activation caused by AML monotherapy is detrimental long-term and thus whether adjunctive treatment with a RAAS-mitigating drug is warranted when treating SH in cats. The effect of nonspecific or balanced RAAS activation might also differ based on presence of concomitant diseases that could affect baseline RAAS activity, such as cardiomyopathy, hyperthyroidism, or chronic kidney disease, as well as treatments for those diseases.[11,15,18,28] However, in previous studies of AML treatment in cats with naturally-occurring systemic hypertension, no clinically relevant changes in creatinine or electrolytes occurred during treatment periods of 1-4 months.[7,11,29]

The present study adds to the existing information regarding the PK of AML in cats derived from both peer-reviewed reports[30] and data obtained to support drug registration with the Food and Drug Administration, performed in accordance with Veterinary International Conference on Harmonization guidelines. Compared to these previous reports, this study provides the most comprehensive description of amlodipine disposition kinetics in healthy cats. Non-compartmental analysis estimates of AML average concentrations in our study align with a prior communication by Elliott et al.[31] Additionally, the typical plasma elimination half-life observed in our study agrees with findings from previous research in humans and dogs,[32] albeit slightly shorter than the duration stated in the summary of product characteristics.[16]

A limitation of this study was the use of healthy cats presumed to have normal baseline RAAS activity, rather than cats with naturally occurring SH or experimentally-induced RAAS activation. Indeed, AML did not significantly decrease BP in these healthy cats, suggesting intact physiologic mechanisms to preserve BP (including RAAS); however, this finding might also reflect inherent variability and imperfection in noninvasive BP techniques[1] or the fact that BP was measured at the pharmacokinetic trough of AML. By providing a rich database of PK/PD information in healthy cats treated with AML, this study serves as a starting point for further data integration and hypothesis testing regarding treatment of systemic hypertension in cats.

Additional limitations of this study include the fact that only AML monotherapy was studied, and therefore we are unable to compare the effects of AML on RAAS activity to the effects of other antihypertensive medications (ACE inhibitors, angiotensin receptor blockers) or combinations of these medications. Diet (and therefore sodium consumption) was not standardized in this study, and differences in diet composition might have resulted in inter-cat variability in RAAS pharmacodynamics. To counteract this variability, all cats received their typical diet at consistent times throughout both treatment periods (AML and placebo), and owners were instructed not to give sodium-containing treats during the study. The stress of hospitalization and repeated blood sampling, as well as the use of gabapentin to decrease this stress and facilitate sample collection, could have affected RAAS activity in our study cats; however, this effect should have been minimized with our placebo-controlled crossover design, and no change from baseline in BP was noted in the placebo group. While gabapentin is not expected to have a PK interaction with AML,[33] a direct effect of gabapentin on the RAAS or pharmacodynamic interaction with AML is possible and was not directly investigated in this study. Some cats might have had subclinical RAAS-modulating systemic diseases not detectable on routine screening diagnostic tests. Finally, only circulating RAAS metabolites were studied, whereas local renal tissue RAAS concentrations might be more relevant in the pathophysiology of SH.[34]

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## CONFLICT OF INTEREST DECLARATION

Emilie Guillot is the Global Technical Manager (Cardiology, Nephrology, and Hypertension) for the study sponsor, Ceva Sante Animale. Drs. Ward and Mochel have served as consultants for Ceva Sante Animale and have received reimbursement and honoraria for consulting, expert testimony, travel, and service as key opinion leaders (KOLs). Although Ceva Sante Animale provided funding for the project and approved study design, Ceva was not involved in data collection or statistical analysis of results. Oliver Domenig is an employee of Attoquant Laboratories, the commercial laboratory that performed RAAS fingerprint analysis for the study. Attoquant was not involved in sample collection or statistical analysis of results. No other authors declare a conflict of interest.

## OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

## INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Approved by Iowa State University IACUC, number 21-025.

## HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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