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Pharmacokinetic and pharmacodynamic properties of orally administered torasemide in healthy cats

Marine Roche-Catholy[1] | Dominique Paepe[1] | Mathias Devreese[2] | Bart J. G. Broeckx[3] | Frederique Woehrlé[4] | Marc Schneider[4] | Andrea Garcia de Salazar Alcala[5] | Arnaut Hellemans[1] | Pascale Smets[1]

[1]Small Animal Department, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

[2]Department of Pathobiology, Pharmacology and Zoological Medicine, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

[3]Laboratory of Animal Genetics, Ghent University, Faculty of Veterinary Medicine, Merelbeke, Belgium

[4]Global Drug Development Division, Lure, France

[5]Avogadro LS, Fontenilles, France

Correspondence
Pascale Smets, Small Animal Department, Ghent University, Faculty of Veterinary Medicine, Salisburylaan 133, 9820 Merelbeke, Belgium.
Email: pascale.smets@ugent.be

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Vetoquinol

Abstract

Background: In people and dogs, torasemide has higher bioavailability, longer half-life, and longer duration of action than equivalent doses of furosemide but data regarding pharmacological properties of torasemide in cats are limited.

Objective: To assess pharmacokinetic and pharmacodynamic parameters of torasemide in healthy cats, and to investigate the effects of a single administration of torasemide on indicators of diuresis, plasma creatinine concentration, blood pressure, electrolyte concentrations and markers of the renin-angiotensin-aldosterone system (RAAS).

Animals: Six clinically healthy adult European shorthair cats.

Methods: Randomized 4-period crossover design with 3 groups and 4 treatments. Pharmacokinetic parameters were obtained using a noncompartmental analysis, and the clinically effective dose was assessed using a Hill model.

Results: Mean absolute bioavailability was estimated at 88.1%. Mean total body clearance was 3.64 mL/h/kg and mean terminal half-life was 12.9 hours. Urine output significantly increased after torasemide administration ($P < .001$). The urine sodium : potassium ratio (uNa : uK) paralleled and was statistically correlated to urine output ($P < .001$). Administration of a single torasemide dose led to a significant dose-dependent increase in urine aldosterone : creatinine ratio (uAldo : C; $P < .001$) and a transient decrease in plasma potassium concentration ($P < .001$) but did not affect blood pressure or plasma creatinine concentration.

Conclusions and Clinical Importance: A single torasemide dose leads to a significant increase in diuresis and renin-angiotensin-aldosterone system (RAAS) activation in healthy cats, with high absolute bioavailability, and without clinically relevant adverse

Abbreviations: %*D*, fraction of the dose eliminated in urine; AUC_{INF}, area under the concentration-time curve extrapolated to infinity; CHF, congestive heart failure; Cl, chloride; Cl_R, renal clearance; C_{max}, maximal plasma concentration; *D*, actual administered dose; *E*, excreted urine volume in 24 hours; E_{max}, maximum urine volume excreted in 24 hours; EX_{50}, eliminated urinary amount of torasemide which induces an excreted urine volume of half of the E_{max}; *F*, absolute bioavailability; F_u, absolute bioavailability with urine data; HDO, high definition oscillometry; HF, heart failure; K, potassium; MRT_{INF}, mean residence time extrapolated to infinity; Na, sodium; pCr, plasma creatinine; RAAS, renin-angiotensin-aldosterone system; $T_{½,z}$, mean terminal half-life; T_{max}, Time to reach maximum plasma concentration; uAldo : C, urine aldosterone : creatinine ratio; uCr urinary, creatinin; USG, urine specific gravity; V_{ss} mean, volume of distribution; X_u total, eliminated urinary amount of torasemide in 24 hours; X_u^{∞}, total eliminated urinary amount of torasemide in 24 hours extrapolated to infinity.

effects. Pharmacokinetic parameters indicate that once daily dosing of 0.27 mg/kg may be appropriate in a clinical setting.

KEYWORDS
congestive heart failure, diuretic, feline, hypertrophic cardiomyopathy

1 | INTRODUCTION

Congestive heart failure (CHF) is a complex clinical syndrome associated with chronic cardiac dysfunction that causes excessive cardiac filling pressures and is characterized by sodium (Na) and fluid retention, resulting in edema. Furosemide, a loop diuretic inducing urinary excretion of Na and potassium (K) and an increase in urine volume, currently is recommended as primary treatment for the management of CHF in human and veterinary patients.[1-3] However, as a result of decreased glomerular filtration rate, increased Na reabsorption and activation of the renin-angiotensin-aldosterone system (RAAS), resistance to furosemide has been described in humans and animals, requiring progressive dose increments to maintain the same level of diuresis,[4,5] highlighting the need to explore therapeutic alternatives to furosemide.[6]

Torasemide shares a similar site of action with other loop diuretics,[7] and has higher bioavailability, longer half-life, and longer duration of action than furosemide in people,[8,9] leading to lower mortality rate and greater improvement in CHF class compared to administration of furosemide and other diuretics.[9]

Although torasemide has proven safe and effective with sufficient PO absorption in dogs, cats, and rats,[3,10-15] limited data is available regarding the pharmacokinetic and pharmacodynamic properties of torasemide for applications in veterinary cardiology. Experimental studies in small groups of dogs confirm the longer half-life, longer duration of action, and higher bioavailability of torasemide compared to furosemide.[3,5,10,15,16] In healthy experimental dogs, torasemide used at 5% to 10% of the dose of furosemide produced equivalent diuresis.[3,17] In several clinical trials performed in dogs with degenerative mitral valve disease, torasemide was found to be noninferior to furosemide, and significantly decreased the risk of cardiac-related death and worsening of CHF class.[18,19]

The long half-life of torasemide allows once daily dosing in dogs, which is of even greater interest in cats, a species known to be often difficult to medicate, and represents an interesting alternative to furosemide administered q12h to q8h. A previous study concluded that the diuretic effect of torasemide peaked 4 hours after administration (vs 2 hours with furosemide) and persisted for 12 hours (vs 6 hours) in cats with experimentally-induced left ventricular hypertrophy.[3] A recent retrospective study identified good tolerability of torasemide in cats with spontaneous CHF at a median dosage of up to 0.29 mg/kg q24h (range, 0.16-0.40).[20] Nevertheless, fundamental data on the basic pharmacological properties of torasemide in cats still is lacking, and is essential for the design of prospective clinical trials in this species. Thus, our objectives were to assess the pharmacokinetic and pharmacodynamic parameters of torasemide in healthy cats and to study the effects of a single administration of torasemide on indicators of diuresis, plasma creatinine concentration, blood pressure, electrolyte concentrations, and markers of RAAS activation.

2 | ANIMALS, MATERIALS, AND METHODS

2.1 | Cats and summary of the study design

The study plan was approved by the local ethical committee (Avogadro LS Animal Ethics Committee, authorization #D 31188 01) and animal housing and care complied with the recommendations of Directive 2010/63/EU. Six adult purpose-bred European cats, determined to be healthy by physical examination, CBC, plasma biochemistry profile, urinalysis, noninvasive blood pressure measurement and echocardiographic examination, were included in the study. They consisted of 3 males and 3 females, with a mean age of 4.5 years (range, 3.9-4.8) and mean body weight of 4.27 kg (range, 4.02-4.51). After a period of acclimatization upon arrival in the facility, cats are placed in group accommodation to socialize them and then are trained several times a week to be placed on a table, to be brushed, to be weighed, and to be gently restrained for blood collection. Once they appear fully at ease with the operators and the process, they can be included in experimental studies, provided they are acclimatized to the required accommodation 2 weeks beforehand. Cats were group-housed during the wash-out periods and kept in individual cages during the sampling periods. They were fasted 12 hours before torasemide administration and fed commercial dry food 4 hours after diuretic administration (Royal Canin Fit 32, see supplementary material for composition, Data S1). No dietary changes were made during the course of the study. Water was provided ad libitum.

The study was performed according to a randomized crossover design with 4 periods, 4 treatments and 3 groups of 2 animals (Figure 1). The animals were randomized to 1 of the 3 different PO doses of torasemide (0.1 mg/kg, 0.2 mg/kg and 0.4 mg/kg, respectively) for the first 3 periods (Upcard 0.75 mg or 3 mg, Vetoquinol). The randomization took place on day 1, using the software R (version 3.5.2 "Eggshell Igloo"). During the fourth period, all animals were allocated to the same group, and given torasemide at a dosage of 0.2 mg/kg IV (UPED P3G1 torasemide 0.1%, Vetoquinol). A 2-week washout period (approximately 32 half-lives of torasemide)[17] was used between treatment periods. For PO administration, the tablets were cut and weighed depending on the body weights of the cats to ensure dose accuracy. After PO administration, 1 to 2 mL of water

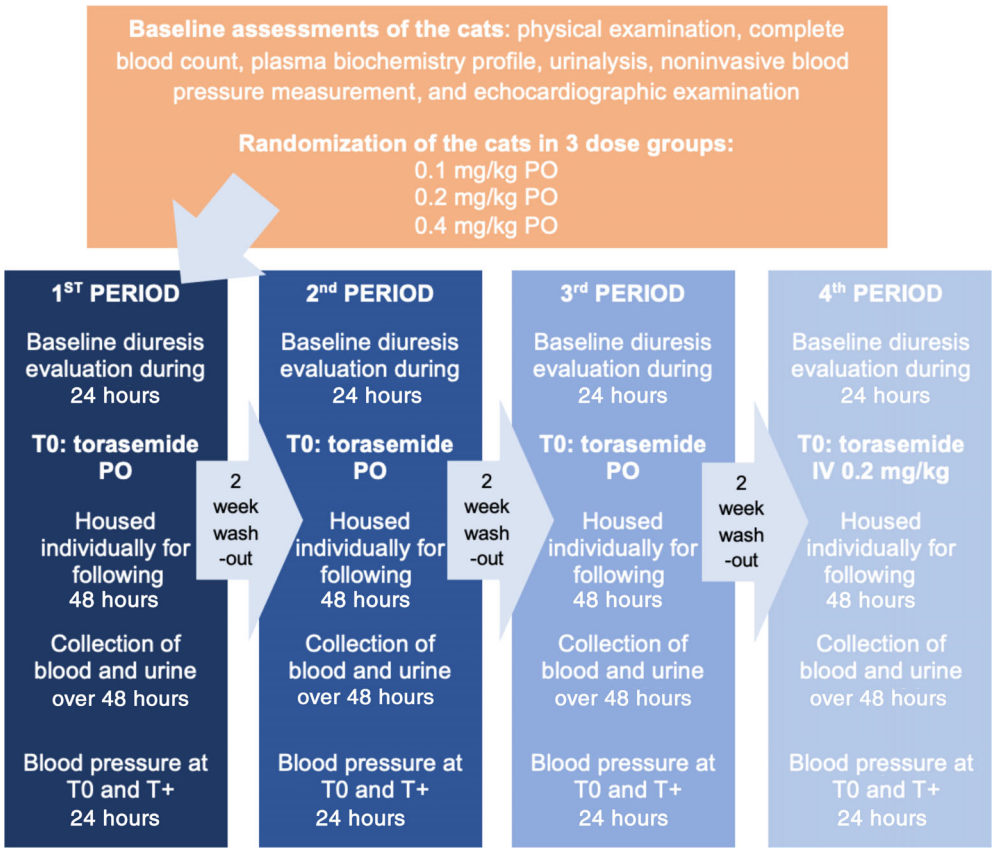

FIGURE 1 Schematic representation of the 4 periods of the study (n = 6 cats)

were administered to ensure esophageal clearance. Investigators were not blinded to treatment groups. Complete physical examination including evaluation of clinical markers of dehydration (mucous membrane moisture, skin tent response, eye position, and corneal moisture) as previously described[21] was performed daily on each cat during the sampling periods.

2.2 | Assessment of urine output

Before the first period, evaluation of each cat's baseline urine output was performed. On day 1, exactly 24 hours before the first torasemide administration, the bladder of each cat was emptied by means of ultrasound-guided cystocentesis using a 22G needle, and urine emptying was confirmed by ultrasonography (Vivid iQ, GE, USA). Urine output then was assessed over the next 24 hours using a specific cat litter that allows for urine collection (Catrina perle litter, Kruuse A/S, Ref. 275021). At the end of the 24-hour period, the bladder again was emptied as described above, before the first diuretic administration. Total 24-hour baseline urine output was defined as the combined volume of urine collected from the litter and the volume of urine removed from the bladder by the second cystocentesis.

To ensure adequate collection of the 24-hour urine volume in each study period, the bladder of each cat was emptied using ultrasound-guided cystocentesis as described above, just before torasemide administration (T0). After torasemide administration, urine output was assessed during 24 hours as described above using specific cat litter and by emptying the bladder using ultrasound-guided cystocentesis at the end of the 24-hour period (T24h). The cats were kept in their individual cages with access to the specific litter described above for the remainder of the sampling period (until T48h). The urine volume collected was indexed to body weight and divided by the time between the time of collection and the last timepoint of urine production, yielding urine output in mL/kg/h. The diuretic effect was defined as a urine output higher than baseline.

2.3 | Blood pressure, blood, and urine sampling for analysis

The cats were weighed daily. Blood pressure was measured in each cat at baseline, at T1h and T24h, with a tail cuff using an oscillometric monitor device (High Definition Oscillometry; HDO) as previously described.[22]

Blood (2.3 mL) was collected before treatment (T0) and at 15, 30, and 60 minutes (T15m, T30m, and T60m, respectively), and 2, 4, 6, 8, 24, and 48 hours (T2h, T4h, respectively) after torasemide administration in periods 1, 2, and 3. In period 4 (IV administration), an additional sampling was performed at T5 min. Samples of blood were divided into 2 tubes: 0.5 mL was placed in K_2EDTA tubes and directly stored at +5°C until analysis, and 1.7 mL was placed in lithium heparin tubes, placed on ice and rapidly processed (centrifugation at 2000*g* for 10 min at +5°C). The plasma from each vacutainer was divided into 3 aliquots of 250 µL each, and stored at −80°C until shipping for analysis. On each blood sample, torasemide concentration, packed cell

volume (PCV), sodium (Na) and potassium concentrations (K) were measured. On the samples collected at T0 and T48h, chloride (Cl) and plasma creatinine concentrations (pCr) also were assessed, in addition to the aforementioned analyses.

Similarly, urine was collected by cystocentesis at T0 and T24h, and with the use of the specific cat litter at prespecified windows: 0 to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 24, 24 to 36, and 36-48 hours. Urine weight and volume were recorded immediately after collection. For each urine sample, 1 mL was stored at +5°C, 4 aliquots of at least 300 μL were stored at −80°C, 1 mL was centrifuged for 5 minutes at 2000*g* and 800 μL of the supernatant was stored at −80°C until analysis. For each sample, urine specific gravity (USG), urine Na (uNa) and K (uK) concentrations, torasemide, as well as urinary creatinine concentration (uCr) and aldosterone (uAldo) concentrations were assessed. Additionally, urinary Cl concentration (uCl) was measured in the baseline cystocentesis sample and the 36 to 48 hours samples. To correct for urinary dilution, uNa and uK were indexed to uCr. They also were used to calculate the uNa : uK ratio, a short-term marker of RAAS activation.[21] Similarly, uAldo was indexed to uCr to calculate the uAldo : C ratio, an additional indicator of RAAS activity.

Plasma and urine concentrations of torasemide were determined using a high performance liquid chromatography (HPLC-MS/MS) method[10] and uAldo was determined by radioimmunoassay[23] (see supplementary material, Data S2). The uCr was measured by enzymatic photometric determination. Electrolyte concentrations and pCr were measured using an autoanalyzer (ADVIA1800, Siemens). Electrolytes concentration reference intervals were 150 to 165 mmol/L for Na, 3.5 to 5.8 mmol/L for K, and 112 to 129 mmol/L for Cl.

2.4 | Pharmacokinetic and pharmacodynamic modeling

Individual plasma data sets were used to perform a noncompartmental analysis using Phoenix WinNonlin software (version 6.3). Classical pharmacokinetic parameters were calculated. Absolute bioavailability (*F*) was calculated using data obtained after the single PO and IV doses of 0.2 mg/kg, using the following equation:

$$F = (\mathrm{AUC_{INF}PO}/\mathrm{AUC_{INF}IV}) \times (\mathrm{DIV}/\mathrm{DPO}) \times 100.$$

where $\mathrm{AUC_{INF}}$ is the area under the concentration-time curve extrapolated to infinity and D the actual administered dose.

The individual dose-corrected C_{max} and $\mathrm{AUC_{INF}}$ values were obtained after the PO doses were tested for proportionality using analysis of variance followed by regression analysis. For C_{max} and $\mathrm{AUC_{INF}}$ to be proportional to the dose, the regression line obtained with the measured values was compared with the regression line obtained with inclusion of the 0 coordinates. The slopes of both regression lines should not be significantly different.

Using the urinary concentration of torasemide and the volume of urine excreted during the different time intervals, cumulative excreted amount and excretion rates were calculated. Using the actual administered doses, the fraction of the dose eliminated in urine (%*D*) as unchanged torasemide was calculated. Absolute bioavailability (*F*) also was calculated using urinary data obtained after the single PO and IV doses of 0.2 mg/kg. The following equation was used:

$$F = (X_u^{\infty}\mathrm{PO}/X_u^{\infty}\mathrm{IV}) \times (\mathrm{DIV}/\mathrm{DPO}),$$

where X_u^{∞} is the total amount of torasemide eliminated in urine.

The individual dose-corrected X_u^{∞} values obtained after the PO doses were tested for proportionality using the same procedure used for plasma data.

Using both plasma and urine data, renal clearance ($\mathrm{Cl_R}$) was calculated using the following equation:

$$\mathrm{Cl_R} = X_u^{\infty}/\mathrm{AUC_{INF}}.$$

Regarding pharmacodynamics, diuresis in mL/h was plotted against excretion rates using the midpoint of the collection time intervals (Figure 2). A clockwise hysteresis loop was obtained, confirming the occurrence of a fast tolerance phenomenon. Thus, a direct pharmacokinetic-pharmacodynamic (PKPD) model cannot be used for the simulation of diuresis including a time factor. However, using a fixed 24-hour period after administration, the excreted urine volume and eliminated Na amount were plotted against the total eliminated torasemide amount in urine. A Hill E_{max} model was used for both parameters rather than a power model because it fitted the data the best, using the following equation:

$$E = (E_{max} \times X_u^{\gamma})/(\mathrm{EX}_{50}^{\gamma} + X_u^{\gamma}),$$

where *E*: excreted urine volume in 24 hours or eliminated Na amount in 24 hours, E_{max}: maximum urine volume or maximum Na amount

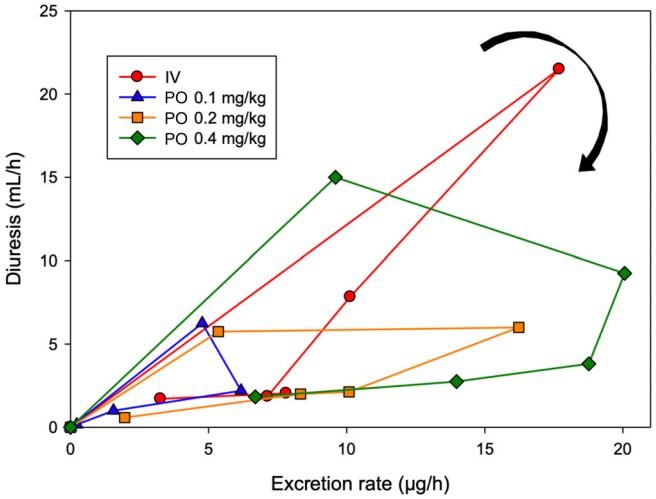

FIGURE 2 Clockwise hysteresis loop of diuresis vs excretion rate of torasemide in cat 3 after a single administration of an IV dose of 0.2 mg/kg and of PO doses of 0.1, 0.2 and 0.4 mg/kg

excreted in 24 hours, X_u: total eliminated urinary amount of torasemide in 24 hours, EX_{50}: eliminated urinary amount of torasemide that induces an excreted urine volume or Na amount of half of the E_{max}, γ: coefficient of Hill. The total excreted urine volume during a 24-hour period after administration was divided by the total excreted torasemide amount during this period to calculate the "efficacy" in mL/μg of torasemide for each administered dose.

2.5 | Statistical analysis

Additional statistical analyses were performed using R version 3.5.2 (Eggshell Igloo). Significance was set at $\alpha \leq .05$. To evaluate the general diuretic effect of torasemide, regardless of dose, the urine volume and urinary output pre- and postadministration of torasemide (dependent variable) were compared using a mixed model with "animal" as random effect and the factor "treatment" as fixed effect. Mixed models with "animal" as random effect and "time" as fixed effect were used to compare the baseline of several parameters with results observed at different time points after treatment. The dependent variables were blood pressure, urine output, uNa : uK ratio, USG, uAldo : C ratio, plasma K, Na, Cl, and pCr. These analyses were performed on the entire dataset to evaluate the global effect of treatment and on subsets for each treatment dose. In a final analysis, a mixed model with "animal" as random effect was used to evaluate the association between uNa : uK ratio (dependent variable) and urine output, and between USG (dependent variable) and urine output. Significance of fixed effects was assessed using likelihood ratio tests. *P*-values were corrected using the Bonferroni method to adjust for multiple testing and presented as such in the results.

3 | RESULTS

Two cats vomited during the course of the study. A preanalytic evaluation of the plasma torasemide concentration confirmed that these 2 cats did not properly receive their torasemide doses in the 0.2 mg/kg

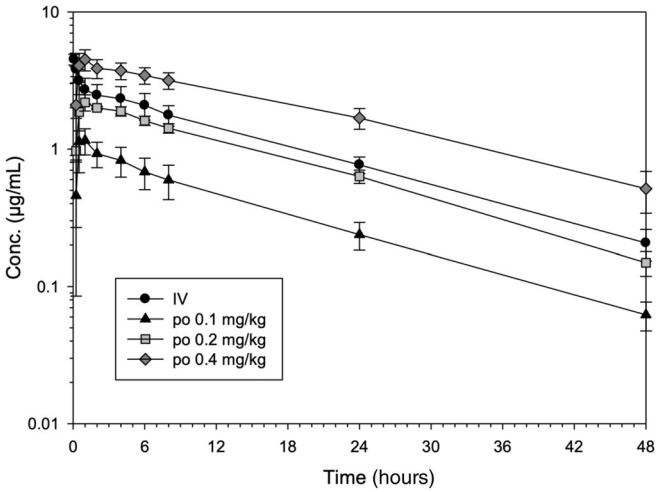

FIGURE 3 Mean concentration-time profiles of torasemide in plasma of cats after single administrations of an IV dose of 0.2 mg/kg and of PO doses of 0.1, 0.2 and 0.4 mg/kg

Parameter	IV 0.2 mg/kg	PO 0.1 mg/kg	PO 0.2 mg/kg	PO 0.4 mg/kg
D (mg/kg)	0.20 (0.01)	0.09 (0.004)	0.18 (0.01)	0.36 (0.02)
C_{max} (μg/mL)	na	1.21 (0.24)	2.38 (0.24)	4.66 (0.70)
T_{max} (h)	na	0.67 (0.26)	1.00 (0.71)	0.75 (0.27)
$T_{½\lambda z}$ (h)	12.9 (1.76)	12.1 (0.86)	12.2 (0.69)	15.3 (2.14)
AUC_{INF} (μg.h/mL)	55.1 (7.53)	17.8 (4.15)	42.4 (4.24)	106 (18.6)
F (%)	na	na	88.1 (18.4)	na
Cl (mL/h/kg)	3.64 (0.59)	na	na	na
V_{ss} (L/kg)	0.061 (0.011)	na	na	na
MRT_{INF} (h)	17.0 (2.5)	16.0 (1.2)	17.4 (1.5)	21.0 (3.0)
X_u^{∞} (μg/kg)	72.6 (20.5)	25.0 (11.9)	68.4 (21.7)	141 (28.6)
Cl_R (mL/h/kg)	1.33 (0.400)	na	na	na
F_u (%)	na	na	104 (12.4)	na
%*D* (%)	37.3 (11.3)	32.4 (8.34)	39.5 (13.8)	40.1 (8.69)
Efficacy (mL/μg)	0.28 (0.126)	0.16 (0.0997)	0.23 (0.0666)	0.25 (0.0640)

TABLE 1 Pharmacokinetic parameter estimates of torasemide in cats following a single oral (0.1, 0.2, and 0.4 mg/kg) and IV (0.2 mg/kg) administration

Abbreviations: %*D*, fraction of the dose eliminated in urine; AUC_{INF}, area under the concentration-time curve extrapolated to infinity; Cl, clearance; Cl_R, renal clearance; C_{max}, maximal plasmatic concentration; *D*, actual administered dose; *F*, absolute bioavailability; F_u, absolute bioavailability with urine data; MRT_{INF}, mean residence time extrapolated to infinity; na, not available; $T½\lambda z$, mean terminal half-life; T_{max}, time to reach maximum plasmatic concentration; V_{ss}, mean volume of distribution; X_u^{∞}, total eliminated urinary amount of torasemide in 24 h extrapolated to infinity.

PO group, (ie, low plasma torasemide concentrations). These cats subsequently were removed from the analyses.

3.1 | Pharmacokinetics

The obtained mean parameters are given in Table 1. After IV administration, mean total body clearance (Cl) was estimated at 3.64 mL/h/kg and mean terminal half-life ($T_{½λz}$) was 12.9 hours. The fraction of the administered dose eliminated in urine (%*D*) as unchanged torasemide was 37.3%. Renal clearance was 1.33 ± 0.40 mL/h/kg, corresponding to 36.5% of the total body clearance.

After PO administration, mean C_{max} increased proportionally to the dose administered ($P = .78$ for the slope and $P = .94$ for the intercept) from 1.21 μg/mL (dose of 0.1 mg/kg) to 4.66 μg/mL (dose of 0.4 mg/kg). The AUC_{INF} values also increased dose dependently, but the increase was not proportional to the dose ($P = .02$ for the slope and $P = .02$ for the intercept). The T_{max} values were consistent for the 3 different doses. Mean absolute bioavailability calculated using plasma data was 88.1%. The fraction of the administered dose eliminated in urine as unchanged torasemide ranged between 32.4% (dose of 0.1 mg/kg) and 40.1% (dose of 0.4 mg/kg). Absolute bioavailability calculated using urine data was 104%. The amount of torasemide eliminated in urine also increased proportionally to the dose ($P = .57$ for the slope and $P = .76$ for the intercept) from 25.0 μg/kg (dose of 0.1 mg/kg) to 141 μg/kg (dose of 0.4 mg/kg). Mean concentration-time profiles of torasemide in plasma and mean cumulated amount-time profiles of torasemide eliminated in urine are presented in Figures 3 and 4, respectively.

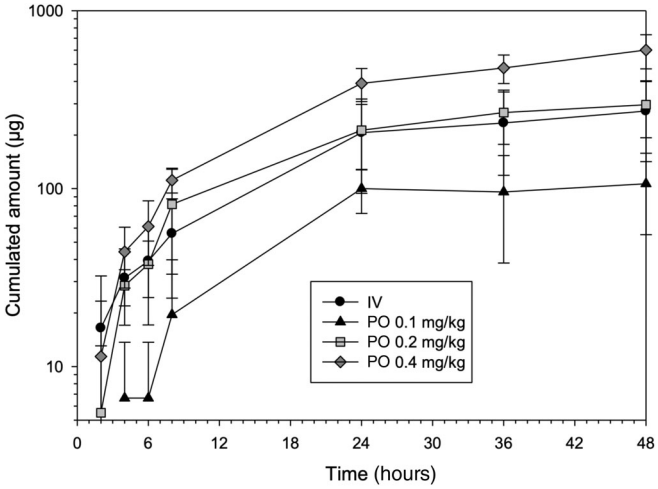

FIGURE 4 Mean cumulated amount-time profiles of torasemide eliminated in urine of cats after single administration of an IV dose of 0.2 mg/kg and of PO doses of 0.1, 0.2 and 0.4 mg/kg

3.2 | Pharmacodynamics

3.2.1 | Hill modeling

The Hill E_{max} plot of the total volume of urine excreted in 24 hours vs the total amount of torasemide eliminated in urine in 24 hours resulted in a relationship with a correlation coefficient of 0.84, which was significant ($P < .0001$; Figure 5A). A similar relationship was observed with the total amount of Na excreted in 24 hours (correlation coefficient, 0.87; $P < .0001$; Figure 5B). The calculated pharmacodynamics parameters were an E_{max} of 24.3 ± 6.77 mL/kg of urine and 4.58 ± 1.49 mEq/kg of Na in 24 hours, an EX_{u50} of 45.5 ± 15 μg/kg of torasemide in 24 hours with urine volume and 44.6 ± 22.4 μg/kg with Na amount, and a coefficient of Hill of 2.24 ± 0.995 with urine volume and 1.52 ± 0.636 with Na amount. The efficacy of the administered doses, defined as the volume of urine (mL) eliminated in 24 hours induced by 1 μg of torasemide

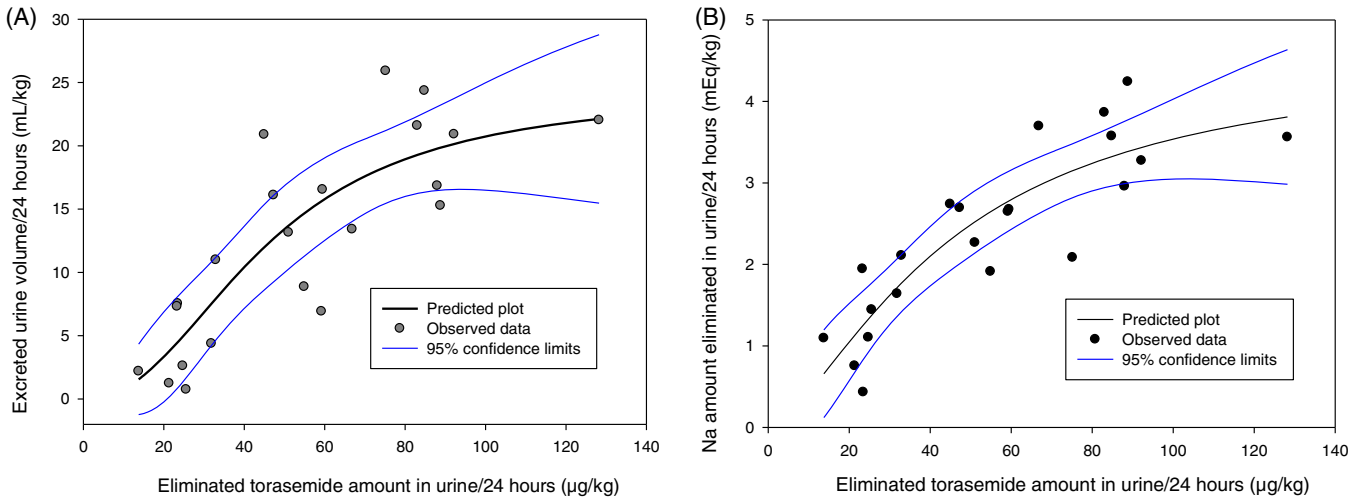

FIGURE 5 Hill Emax plot between excreted urine volume in 24 hours and eliminated torasemide amount in urine in 24 hours (A) and between excreted sodium amount in 24 hours and eliminated torasemide amount in urine in 24 hours (B) after single administrations of an IV dose of 0.2 mg/kg and of PO doses of 0.1, 0.2 and 0.4 mg/kg

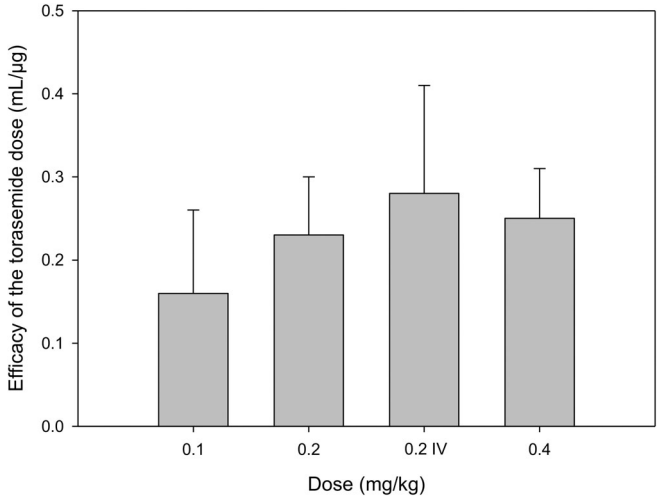

FIGURE 6 Efficacy of torasemide amount eliminated in urine in inducing diuresis in cats after single administrations of an IV dose of 0.2 mg/kg and of PO doses of 0.1, 0.2 and 0.4 mg/kg

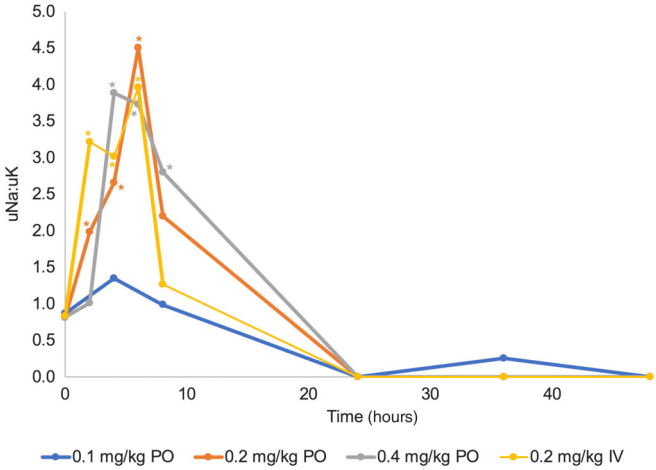

FIGURE 7 Evolution of the mean uNa : uK ratio after torasemide administration in the 4 subgroups. The presence of a significant difference in uNa : uK between a specific timepoint and baseline within the same dose group is represented by the use of an asterisk symbol (*)

is shown in Figure 6. The highest mean efficacy was 0.28 mL of urine excreted/µg of torasemide eliminated in urine and was obtained after the IV dose of 0.2 mg/kg. The PO dose of 0.4 mg/kg induced a lower efficacy with a mean value of 0.25 mL/µg.

3.3 | Urine volume and USG

Compared to baseline (T0), urine output was significantly increased after torasemide administration ($P < .001$). Similarly, torasemide administration led to a significant decrease in USG ($P < .001$), lasting 8 hours after administration at the 0.1 and 0.2 mg/kg PO doses ($P = .001$ and $P < .001$), and 24 hours after administration at the PO dose of 0.4 mg/kg ($P < .001$).

3.4 | Urinary electrolytes and uAldo : C

The uNa : uK followed a similar pattern to urine output, with an overall increase compared to baseline persisting for 8 hours ($P < .001$), and varying depending on the dose administered from 6 hours for 0.2 mg/kg ($P < .001$) to 8 hours for 0.4 mg/kg ($P < .001$; Figure 7). A separate statistical analysis confirmed the significant association between this ratio and calculated urine output ($P < .001$). Assessing urinary electrolytes corrected with uCr individually, a marked increase in uNa was observed over the 24 hours after torasemide administration, whereas uK remained relatively stable over time (Figure 8).

Torasemide administration led to a significant increase in uAldo : C ($P < .001$). In the mixed model with "time" as fixed effect, the significant increase in uAldo : C was only present starting from T24h ($P < .001$) and lasted until the T48h sample ($P < .001$). At the 0.1 mg/kg PO dose, a significant increase was present only in the T24h sample ($P = .03$). At the 0.2 mg/kg PO dose, a significant increase was present

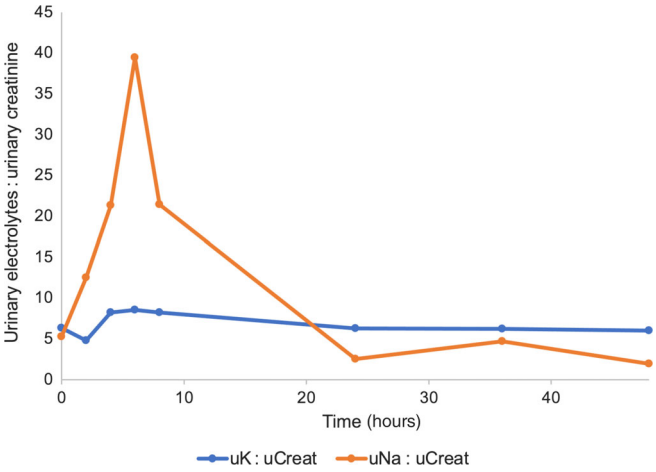

FIGURE 8 Evolution of the mean urine electrolyte-to-urine creatinine ratio for potassium (uK : uCreat) and sodium (uNa : uCreat) after torasemide administration in all subgroups combined

only in the T24h and T48h samples ($P < .001$ and $P = .009$). Finally, at the 0.4 mg/kg PO dose, a significant increase was observed for the T24h sample ($P = .02$; Figure 9).

3.5 | Plasma electrolytes

Torasemide administration led to a significant decrease in plasma K ($P < .001$; Figure 10). In the IV group, the K decrease started at T4h and lasted until T8h. Five of 6 cats had plasma K below the reference interval at ≥1 timepoints (lowest concentration observed: 3.1 mmol/L). For the PO subgroups of 0.2 mg/kg or 0.4 mg/kg, the mixed model identified a significant decrease in K only in the T6h sample ($P = .04$ and $P = .003$ for each dose, respectively). One and 2 cats showed

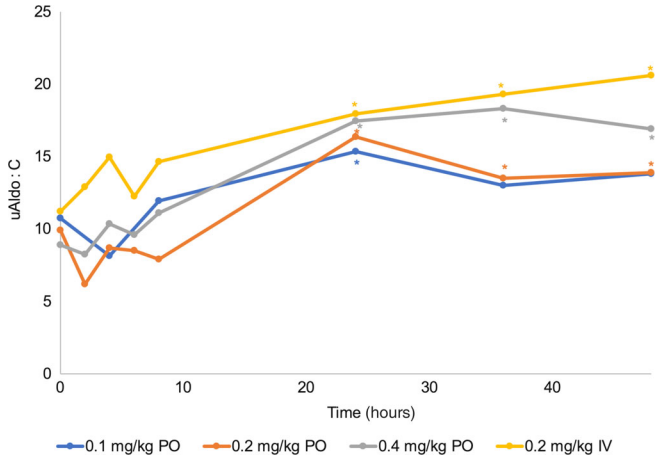

FIGURE 9 Evolution of the uAldo : C ratio after torasemide administration in the 4 subgroups. The presence of a significant difference in uAldo : C between a specific timepoint and baseline within the same dose group is represented by the use of an asterisk symbol (*)

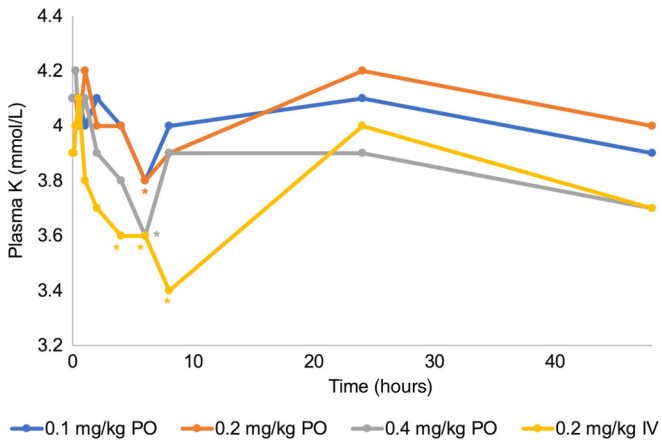

FIGURE 10 Evolution of the plasma potassium after torasemide administration in the 4 subgroups. The presence of a significant difference in plasma potassium between a specific timepoint and baseline within the same dose group is represented by the use of an asterisk symbol (*)

hypokalemia at this timepoint in these 2 subgroups, respectively (lowest concentration observed: 3.3 mmol/L). For the 0.1 mg/kg PO dose, no significant difference in K was observed at any timepoint ($P = .52$). Two cats experienced hypokalemia at 1 timepoint, T6h and T8h, respectively (3.3 mmol/L).

Torasemide administration also was associated with a mild but significant ($P < .001$) decrease in plasma Na. In the 0.2 mg/kg dose subgroup after PO and IV administration, this significant difference only was identified at T6h ($P = .03$ and $P < .001$ for each administration route, respectively). In the 0.4 mg/kg dose subset, this significant decrease was only identified at T24 ($P = .02$). For the 0.1 mg/kg dose, no significant difference in Na was observed at any timepoint. Only very mild hyponatremia was observed (lowest concentration 148 mmol/L), mostly in the IV group (3 of 6 cats).

No significant changes in plasma Cl were observed after torasemide administration compared to baseline.

3.6 | Physical examination, blood pressure, and plasma creatinine

No significant abnormalities were noted on physical examination. Most cats exhibited few to no clinical signs of dehydration, limited mostly to tacky mucous membrane and mildly decreased skin turgor, without any cats showing signs of severe dehydration (maximal estimated percentage of dehydration: 8%).

As described above, 2 cats vomited after torasemide administration.

No significant difference was observed for systolic, diastolic, and mean blood pressure before and after torasemide administration ($P = .22$, .1 and .13, respectively). Similarly, no significant differences were identified for pCr ($P = .66$).

4 | DISCUSSION

Single administration of torasemide at dosages of 0.1, 0.2 and 0.4 mg/kg PO and 0.2 mg/kg IV induced significant diuresis, with increased urine output and decreased USG in all cats, without substantial adverse effects. Torasemide was well absorbed PO and rapidly reached therapeutic plasma concentrations similar to those observed in dogs and rats.[3,10-15] The PO bioavailability observed in our study (88%) was similar to that observed in dogs (92%-98%)[10,15] and is markedly higher than the reported bioavailability of furosemide in cats (48 ± 23%)[24] and dogs.[10,15] The total body clearance (3.64 mL/kg/h) was about 3 times lower compared to dogs (7.7-12.4 mL/kg/h[10,15]) whereas the volume of distribution was very similar to that of dogs (61 mL/kg in our study vs 61.9 mL/kg in dogs[10]). Consequently, the terminal half-life was found to be slightly longer than observed in dogs (12.9 hours vs 6-12.3 hours[10,15]), and markedly higher compared to that observed for furosemide in cats (1.22 hours[24]). The fraction of the administered dose eliminated in urine (%*D*) as unchanged torasemide was lower than in dogs.[11] Renal clearance also was found to be relatively low (1.33 mL/h/kg vs 9.96 mL/h/kg in dogs[10]). The occurrence of metabolites in urine and the degree of plasma protein binding of torasemide remain unknown, and warrant further investigation. The higher potency of torasemide compared to furosemide, combined with its slower clearance estimate and longer elimination half-life confirm its suitability for once daily dosing.

The Hill E_{max} plot of the total volume of urine excreted in 24 hours vs the total amount of torasemide eliminated in urine in 2 hours resulted in an Xu_{50} of 45.5 μg/kg. To increase the efficacy to at least 90% of the maximum effect, the Xu_{90} should be calculated, and was estimated at 121 μg/kg. Using the linear regression obtained with the proportionality analysis of the amount of torasemide eliminated in urine, a dose of 0.27 mg/kg was obtained for the elimination of 121 μg/kg of torasemide in 24 hours. The lowest dose of the 95% confidence interval was 0.19 mg/kg. The highest dose could not be calculated because there were not enough high values for the

eliminated torasemide amount. Because the most effective dose for the excretion of urine per μg of eliminated torasemide in urine (ie, torasemide "efficacy") also ranged between the doses of 0.2 and 0.4 mg/kg (Figure 6), a PO dose of approximately 0.27 mg/kg seems to be an appropriate starting point for the establishment of an optimal dosage of torasemide in cats. Our results were obtained using healthy cats. Any change in the health status of the animals (eg, renal or liver impairment) could alter the obtained pharmacokinetic parameters. Interestingly, this dosage is very similar to the PO dosage selected in another study aiming at assessing the effects of torasemide in cats (0.3 mg/kg)[3] as well as the mean starting dosage of 0.21 mg/kg in a retrospective case series of cats in CHF.[20]

Notably, no severe adverse effects were observed after the single administration of torasemide to healthy cats, up to 0.4 mg/kg PO. No significant effect of torasemide administration on pCr or blood pressure was noted. Sporadically, our study identified a significant decrease in plasma K after torasemide administration. For doses equal to or higher than 0.2 mg/kg PO, this decrease reached significance 6 hours after administration but resolved within 2 hours. After IV administration, this decrease persisted for 2 hours, before normalizing. Finally, at a dose of 0.1 mg/kg, no significant effect of torasemide on plasma K was observed. Hypokalemia was observed in a small number of cats, with the lowest concentration being 3.1 mmol/L. These findings confirm that a single administration of torasemide leads to a temporary dose-dependent decrease in plasma K in healthy cats, which quickly resolves after administration. However, our study does not replicate the effect of chronic administration of torasemide nor the possible comorbidities presented by nonhealthy cats, which could exacerbate K loss.

In the absence of therapeutic administration of a loop diuretic, the uNa : uK has been shown to reflect the effects of aldosterone on urinary electrolytes, and has been used as a short-term marker of mineralocorticoid activity.[25,26] Via their interaction with the $Na^+/2Cl^-/K^+$ cotransporter in the ascending limb of Henle, loop diuretics lead to excretion of Na and K, which alters the uNa : uK ratio, making interpretation of this marker difficult in our setting. We observed that the increase in this parameter temporally paralleled the increase in urine production. Whether this finding is solely a consequence of the relatively higher excretion of Na compared to K after torasemide administration or caused by an increase in aldosterone production associated with the administration of torasemide is unknown at this time. The latter hypothesis goes against the previous perception that torasemide exerts an antialdosterone effect by binding to the mineralocorticoid receptor.[7] Conversely, torasemide resulted in a similar K excretion profile to furosemide in a study performed on a small group of healthy dogs, reducing support for a mineralocorticoid-receptor blocking capability.[17] Nonetheless, as evidenced by Figure 8, a marked increase in Na excretion was observed during the first 24 hours after torasemide administration, whereas urine K remains relatively stable, suggesting that the uNa : uK ratio is more closely correlated with Na excretion and thus diuresis itself rather than activation of the RAAS in our setting. This conclusion was supported by the additional finding that the uNa : uK ratio was significantly correlated with urine output, which is consistent with a previous study in healthy dogs that received a constant rate infusion of furosemide and with a study in people showing lower uNa : uK ratios in patients with diuretic resistance and inadequate urine production after diuretic administration.[25,27] However, because loss of K is primarily driven by activation of RAAS resulting from the natriuretic effect of torasemide, K loss in the first 24 hours is expected to lag behind that of Na. Longer follow-up is needed to fully explore the effect of torasemide on electrolyte excretion.

The uAldo : C ratio was found to be significantly increased after torasemide administration, which is consistent with previous reports in healthy dogs after furosemide and torasemide administration.[17,23,28] The increase was observed after 24 hours and remained above baseline in a dose-dependent pattern. In the 0.1 mg/kg dose group, only the T24h sample showed a significant increase in uAldo : C, whereas this increase lasted until the T48h sample in the highest dose group. This finding confirms that torasemide administration leads to significant activation of the RAAS, the duration of which appears to be dose-dependent. To the best of our knowledge, our study was the first to assess the changes in the UAldo : C ratio at several timepoints over a 24-hour period after administration of a loop diuretic in cats, showing evidence of peaking RAAS activation 24 hours after administration. Earlier effects on the local rather than the systemic RAAS or earlier changes in plasma aldosterone concentration were not studied and cannot be excluded based on our data.

Our study had some limitations. It was performed in healthy cats and does not replicate the pathophysiological changes accompanying CHF, which affect the magnitude of RAAS activation, electrolyte excretion and diuretic response. Urine collection relied on the use of a cat specific litter and not the placement of urinary catheters, which currently is considered the gold standard for urine output assessment. We tried to mitigate this drawback by performing several cystocenteses to ensure accurate collection of the 24-hour urine output. Our study focused only on single administration, and thus does not account for the consequences of repeated dosing of torasemide in cats. Regarding the pharmacokinetic analyses, our study design might have led to underestimation of the fraction of the administered dose eliminated in urine and of the renal clearance. The actual values may be slightly higher because at the last collected urine sample (T48h) there were still measurable concentrations of torasemide in the urine samples. A longer follow-up collection protocol could have yielded more accurate results. Other limitations of our study included the limited number of cats ($n = 6$) as well as the restricted range of the administered doses (between 0.1 and 0.4 mg/kg). For a more complete understanding, higher doses could have been administered. The use of a crossover design with a small number of individuals represents an additional limitation. We tried to minimize this effect by randomizing treatment order, by using an appropriate washout period and by using a statistical model that took period effects into account. Finally, other than uNa : uK and uAldo : C ratios, we did not evaluate other markers of RAAS activation that could have provided better insight into the neurohormonal adaptations after torasemide administration. Nonetheless, our findings provide necessary information for future studies in cats with CHF.

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CONFLICT OF INTEREST DECLARATION

Authors declare no 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

The study plan was approved by the local ethical committee (Avogadro LS Animal Ethics Committee, authorization #D 31188 01).

HUMAN ETHICS APPROVAL DECLARATION

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

ORCID

Marine Roche-Catholy https://orcid.org/0000-0002-5117-0638
Dominique Paepe https://orcid.org/0000-0003-2919-1712
Bart J. G. Broeckx https://orcid.org/0000-0001-6742-3911
Frederique Woehrlé https://orcid.org/0000-0003-2188-854X
Arnaut Hellemans https://orcid.org/0000-0002-5373-3131

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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