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Comparison between the effects of torsemide and furosemide on the renin-angiotensin-aldosterone system of normal dogs

B.M. Potter, DVM[a], M.K. Ames, DVM[a,*], A. Hess, PhD[b], M. Poglitsch, PhD[c]

[a] *Department of Clinical Sciences, Colorado State University, Campus Delivery 1678, Fort Collins, CO, 80523, USA*
[b] *Department of Statistics, Colorado State University, 102 Statistics Building, Fort Collins, CO, 80523, USA*
[c] *Attoquant Diagnostics GmbH, Campus Vienna Biocenter 5, 1030 Vienna Austria*

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Abstract *Introduction/Objectives:* We hypothesized that torsemide and furosemide, at approximately equipotent dosages (similar diuresis), would have comparable effects on the circulating renin-angiotensin-aldosterone system.
Animals, materials and methods: Six, healthy, middle-aged, male Beagles were randomized to receive torsemide (0.1 mg/kg PO q 12 h), furosemide (2.0 mg/kg PO q 12 h), or placebo for 10 days during three separate periods, separated by a 10-day washout period, in a crossover design. Blood was collected on days 1, 5, and 9 and 24-h urine collection ended on days 2, 6, and 10. After repeated measures analysis and Bonferonni correction, variables with an adjusted $p<0.05$ were investigated further, using Tukey's method.
Results: Twenty-four-hour urine production differed significantly between the diuretics only on day 10, with torsemide causing a 38% greater diuresis than furosemide. There was, however, no significant difference in average 3-day diuresis. There were no significant differences between diuretics in the 24-h urinary excretion of sodium, chloride, or potassium, though furosemide caused less kaliuresis than torsemide. Serum renin, angiotensin II, and aldosterone and the urine

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* Corresponding author.
E-mail address: marisa.ames@colostate.edu (M.K. Ames).

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aldosterone-to-creatinine ratio were significantly increased in the diuretic groups, as compared to placebo on days 5/6 and 9/10. There were no significant differences in these values between diuretics. Creatinine and blood urea nitrogen concentrations rose comparably in the diuretic groups, remaining within reference intervals in all dogs.
Conclusions: At approximately equipotent dosages (20:1), torsemide and furosemide produced comparable renin-angiotensin-aldosterone system activation. Torsemide's similar potassium excretion profile to furosemide decreases support for a hypothesized mineralocorticoid-receptor blocking capability.

Abbreviations

AngI	angiotensin I
AngII	angiotensin II
AngIII	angiotensin III
AngIV	angiotensin IV
Ang(1,5)	angiotensin (1,5)
Ang(1,7)	angiotensin (1,7)
BUN	blood urea nitrogen
CHF	congestive heart failure
Cl^-	chloride
EFWC	electrolyte free water clearance
K^+	potassium
LC-MS/MS	liquid chromatography-mass spectrometry
MR	mineralocorticoid receptor
Na^+	sodium
RAS	renin-angiotensin system
RAAS	renin-angiotensin-aldosterone system
UAldo:C	urine aldosterone-to-creatinine ratio

Introduction

Loop diuretics provide symptomatic relief for patients with congestive heart failure (CHF) and form the cornerstone of its medical management. Furosemide has been, and remains, the diuretic of choice for acute and chronic management of CHF in both humans and animals, since its release 50 years ago [1]. Torsemide has several characteristics that have made it appealing as chronic therapy for CHF. First, torsemide is a more potent diuretic than furosemide, resulting in a greater diuresis and natriuresis as well as *possibly* less kaliuresis in dogs [2–4]. Second, the diuresis induced by torsemide may have a 'smoother' profile with a more gradual increase and therefore a less abrupt urgency to urinate. For both oral and intravenous administration, torsemide's peak diuretic effect is longer lasting than that of furosemide [2,5]. The greater diuresis is therefore achieved not by a greater peak diuresis, but rather by torsemide's longer duration of action, as compared to furosemide, due to a slower urinary excretion rate [3]. In dogs, torsemide's diuretic effect has historically been considered to be about 10 times more potent than furosemide. Closer inspection of urine production in early studies and findings in a recent abstract, however, suggest that this factor is greater than 10 at the dosages studied[d] [4,5]. Finally, although not studied as thoroughly as would be desirable in animals, torsemide's profile in humans indicates that quality of life and survival are enhanced when compared with furosemide [6,7].

Because of torsemide's greater potency and longer diuretic effect, it is now being used with increasing frequency in addition to, or in place of, furosemide in the management of refractory CHF in the dog [8,9]. A recent prospective, reference-controlled, single-blinded study showed that torsemide was an effective, and safe diuretic (dosed once daily) in dogs with naturally occurring myxomatous mitral valve disease and varying chronicity of CHF [10]. However, an unavoidable and undesirable effect of loop diuretic therapy is the activation of the renin-angiotensin-aldosterone system (RAAS) [11,12]. Chronic RAAS activation promotes sodium and water retention, vasoconstriction, and pathologic cardiovascular and renal remodeling. Torsemide and furosemide's effect on RAAS has not been compared at equipotent dosages (i.e. dosages that cause similar amounts of diuresis). The only two studies to date

[d] Schneider M, Abtout S, Bonavaud S, Menard J, Woehrle F. Diuretic equipotency between torsemide and furosemide in healthy dogs. Southern European Veterinary Conference 2015. Barcelona, Spain.

do not use equipotent dosages of these diuretics [4,5]. It has also been hypothesized that torsemide may exert an anti-aldosterone effect via mineralocorticoid receptor (MR) antagonism. Studies that have evaluated the effects of torsemide on kaliuresis, however, have produced conflicting results with one study showing reduced kaliuresis (supports MR antagonism) [4] and another showing increased kaliuresis (supports MR activation) [13].

The study of neurohormonal activation is becoming easier as new hormone quantification assays are developed. The recent development of highly sensitive liquid chromatography—tandem mass spectrometry (LC-MS/MS) assay has led to the creation of a renin-angiotensin system (RAS)-Fingerprint® that quantifies multiple RAS peptides and aldosterone from either blood or tissue samples. The use of equilibrium analysis allows for the quantification of angiotensin metabolites from a serum sample without the use of a protease inhibitor. Serum aldosterone is also measured via LC-MS/MS and contributes to this comprehensive RAAS assessment. The urinary aldosterone to creatinine ratio (UAldo:C) has also proven to reflect RAAS activation in both the laboratory and clinical settings [14—20].

As torsemide is becoming more frequently used in small animal medicine, a better understanding of its effects on RAAS will further inform our pharmacotherapy for heart failure. Therefore, the goal of this study was to compare the magnitude of RAAS activation between the two potent loop diuretics, furosemide and torsemide, administered at approximately equipotent dosages. Additionally, the effects on kaliuresis were compared to indirectly evaluate for an MR antagonizing effect of torsemide.

Animals, Materials, and Methods

Dogs and protocol summary

Six male, purpose-bred laboratory dogs were determined to be healthy by physical examination and minimum database (complete blood count, serum biochemistry, and urinalysis). The dogs were housed individually in runs, except for study days 1, 5, and 9, when they were individually housed in metabolism cages, to allow for 24-h urine collection. Dogs were fed (once daily in the morning) a standard dry diet[e] with a sodium content of 121 mg/100 kcal, and given ad lib access to water throughout the study. Dogs were acclimated to metabolism cages starting 2 weeks before the first treatment period. Throughout the study, dogs were housed in an American Association for Laboratory Animal Care-approved facility with controlled light/dark cycles. The Colorado State University College of Veterinary Medicine Institutional Animal Care and Use Committee approved this study.

A crossover design was used where each dog received all three treatments over three periods in a randomized order. The randomization was performed in a balanced manner such that each of the six possible sequences of treatments was randomly assigned to exactly one of the subjects and each period had two dogs receiving each treatment. The three treatments were placebo (PO q 12 h × 10 days), furosemide (2.0 mg/kg PO q 12 h × 10 days), or torsemide (0.1 mg/kg PO q 12 h × 10 days). A 10-day washout period (approximately 23 half-lives of torsemide) [21] was allowed between treatment periods. Medications were administered at 7 a.m. and 7 p.m. Investigators were not blinded to treatment groups.

Data collection

Systemic blood pressure, body weight, and heart rate were recorded on day −1 (day before initiation of the study), 1, 2, 5, 6, 9, and 10. There was no day zero. After a 5-min acclimatization period, Doppler-determined[f] blood pressure was obtained using the coccygeal artery (tail) with the patient standing, using an appropriately sized cuff, and averaging three consecutive measurements, within 10% of one another. The coccygeal artery was used to allow patients to stand during measurement and reduce stress and changes in body position before obtaining the serum sample for RAAS peptide analysis. Blood pressures were measured between 8 a.m. and 10 a.m. and before sedation.

A physical examination and minimum database (complete blood count, serum biochemistry, and urinalysis) were performed before each of the three treatment periods (day −1). Also, urinalyses were performed on the final day of each treatment period (day 10) and urine cultures were performed on the final day of the study. On days 1, 5, and 9, dogs were sedated with butorphanol (0.2 mg/kg intravenously), and their bladders were emptied via catheterization, which was confirmed via ultrasound. Dogs were then placed in metabolism cages for 24 h. Urine was collected in Styrofoam containers surrounded by an ice bath. Every 4 h, urine was transferred to sterile plastic containers

[e] PMI Nutrition Pet Foods, St. Louis, Missouri, USA.

[f] Parks Medical Electronics, Inc, Aloha, Oregon, USA.

Table 1 Blood pressure, body weight, and heart rate in healthy dogs treated with placebo, furosemide, or torsemide. Values are listed as mean ± standard error of mean (mean [SEM]).

Parameters	Placebo						Furosemide						Torsemide					
	Day 1	Day 2	Day 5	Day 6	Day 9	Day 10	Day 1	Day 2	Day 5	Day 6	Day 9	Day 10	Day 1	Day 2	Day 5	Day 6	Day 9	Day 10
Blood pressure (mmHg)	135 (8.5)	134 (8.6)	134 (5.7)	123 (8.5)	126 (5.5)	129 (5.4)	130 (4.8)	137 (6.8)	132 (7.1)	126 (5.3)	133 (6.7)	132 (8.1)	129 (6.5)	125 (5.0)	128 (4.4)	117 (7.7)	135 (5.5)	142 (6.5)
Body weight (kg)	12.1 (0.5)	12.3 (0.5)	12.1 (0.5)	12.4 (0.5)	12.3 (1.3)	12.4 (0.6)	12.3 (0.6)	12.3 (0.6)	12.2 (0.6)	12.3 (0.6)	12.3 (0.6)	12.2 (0.6)	12.5 (0.6)	12.3 (0.6)	12.3 (0.5)	12.3 (0.6)	12.2 (0.6)	12.4 (0.6)
Heart rate (bpm)	122 (7.6)	95 (5.6)	108 (11.3)	104 (7.4)	109 (7.1)	104 (6.7)	122 (5.5)	92 (11.8)	115 (9.4)	115 (5.4)	122 (5.4)	107 (9.4)	119 (5.9)	100 (9.2)	109 (12.6)	111 (10.6)	118 (12.0)	106 (9.8)

bpm: beats per minute.

and stored at 3 °C until processing and freezing. At the end of the 24-h period (days 2, 6, and 10), dogs were sedated with 0.2 mg/kg butorphanol IV and their bladders were catheterized and emptied. This final volume of urine was added to the pooled 24-h sample, which was mixed with a clean glass rod for 1 min. Ten milliliters of the final catheter-obtained sample was kept separate for analysis of a 'spot' urine sample.

The total 24-h urine volume and weight for each dog were quantified at days 2, 6, and 10. The 24-h urine specific gravity and concentrations of creatinine, sodium (Na^+), chloride (Cl^-), and potassium (K^+) were measured at a veterinary diagnostic laboratory and the absolute concentrations of these electrolytes calculated using a method described by Jeunesse and colleagues [22]. Electrolyte free water clearance (EFWC) was calculated from the serum sodium concentration (single sample taken at the onset of 24-h urine collection period), the 24-h urine sodium and potassium concentrations, and 24-h urine volume, using the formula EFWC = Urine Volume * 1 − ([UNa^+ + UK^+]/Serum Na^+). The remainder of the 24-h urine sample was centrifuged at 3,000 relative centrifugal force for 10 min at 3 °C and the supernatant was stored at −80 °C, until analysis of urine aldosterone concentrations were measured by radioimmunoassay at a veterinary diagnostic laboratory[f]. Urine volumes and electrolytes concentrations were normalized for body weight. Water consumption was also quantified for each dog as mL/kg/day.

On day 1, 5, and 9, 15 mL of blood was drawn via jugular venipuncture, before sedation, between 8 a.m. and 10 a.m. for the analysis of serum urea nitrogen (BUN), creatinine, K^+, Na^+, and Cl^- concentrations, RAS-Fingerprint® (angiotensin I [AngI], angiotensin II [AngII], angiotensin III [AngIII], angiotensin IV [AngIV], angiotensin (1,7), [Ang(1,7)], angiotensin (1,5) [Ang(1,5)]) and aldosterone. All blood samples were centrifuged at 2,000 relative centrifugal force for 20 min at 3 °C and the serum was stored at −80 °C, until analysis. The RAS-Fingerprint® analysis was carried out at a commercial diagnostic laboratory. Renin-angiotensin-aldosterone system hormones were quantified via highly sensitive LC-MS/MS[g]. The assay was performed from blood samples that were not treated with protease inhibition. The use of equilibrium analysis allows for quantification of angiotensin metabolites from a serum sample without the use of a protease inhibitor. Briefly, as angiotensinogen concentrations greatly exceed that of 'downstream' metabolites, there is the constant formation of AngI. As angiotensin metabolizing enzymes, including the angiotensin-converting enzyme (ACE), are still active in the sample and also exceed the concentrations of metabolites, there is an establishment of stable equilibrium levels of downstream metabolites, where formation and degradation rates of peptides are equal.

Urine creatinine concentration was measured via a standard colorimetric assay, and this assay was performed at a veterinary diagnostic laboratory[h]. A radioimmunoassay that has been validated in dogs[i] was used to measure urine aldosterone concentration and this assay was performed at a veterinary diagnostic laboratory[j]. The same RIA assay was used for all analyses and the assay performed per the manufacturer's directions, as described elsewhere [16]. Briefly, this assay measures free aldosterone and one of its two major metabolites, aldosterone β-glucuronide

[g] Attoquant Diagnostics GmbH, Campus Vienna Biocenter 5, Vienna, 1030, Austria.
[h] Colorado State University Veterinary Diagnostic Laboratory Fort Collins, Colorado, USA.
[i] Beckmann Coulter ACTIVE® Aldosterone RIA Indianapolis, Indiana USA.
[j] Michigan State University Veterinary Diagnostic Laboratory Lansing, Michigan, USA.

Table 2 Serum analytes in healthy dogs treated with placebo, furosemide, or torsemide on treatment days 1, 5, and 9. Values are listed as mean ± standard error of mean (mean [SEM]). If the value was below the lower limit of detection, the mean was calculated as one-half the lower limit of detection. The SEM for values where all dogs were below the lower limit of detection is zero.

Serum analyte	Placebo			Furosemide			Torsemide		
	Day 1	Day 5	Day 9	Day 1	Day 5	Day 9	Day 1	Day 5	Day 9
Aldosterone (pM)	12.85 (1.85)	11 (0)	11 (0)	11 (0)	192.42[a,b] (86.32)	86.85[a,b] (40.45)	13.38 (2.38)	361.79[a,b] (141.67)	159.39[a,b] (54.99)
Ang 1-5 (pM)	6.35 (2.26)	5.42 (2.01)	7.68 (3.44)	8.90 (3.05)	60.57[a,b] (18.40)	82.74[a,b] (30.96)	5.07 (1.90)	105.45[a,b] (39.29)	110.55[a,b] (33.04)
Ang 1-7 (pM)	4.79 (2.51)	6.54 (4.37)	4.14 (1.91)	13.53 (6.59)	76.65[a,b] (27.27)	83.91[a,b] (37.47)	3.07 (1.06)	166.96[a,b] (49.24)	146.13[a,b] (48.09)
Ang I (1-10) (pM)	45.45 (17.43)	65.78 (28.22)	46.17 (17.19)	110.63 (52.70)	403.65[a,b] (113.47)	543.96[a,b] (250.10)	37.92 (5.69)	673.18[a,b] (193.21)	677.55[a,b] (185.08)
Ang II (1-8) (pM)	27.41 (10.68)	24.45 (9.76)	19.52 (7.84)	50.42 (20.64)	211.37[a,b] (55.56)	253.12[a,b] (86.58)	15.77 (3.04)	357.95[a,b] (90.19)	337.56[a,b] (76.28)
Ang III (2-8) (pM)	2.44 (0.19)	2.68 (0.43)	2.51 (0.26)	4.46 (1.28)	16.31 (6.28)	17.06 (9.10)	2.25 (0)	28.67 (11.04)	24.36 (9.33)
Ang IV (3-8) (pM)	4.31 (1.76)	3.50 (1.28)	4.33 (1.32)	8.00 (3.34)	35.27[a,b] (13.03)	39.01[a,b] (16.85)	2.84 (0.50)	57.00[a,b] (18.85)	42.36[a,b] (16.96)
BUN (mg/dL)	15.17 (1.30)	13.67 (1.45)	13.67 (1.28)	16.17 (1.66)	15.00 (1.97)	16.17 (1.89)	14.83 (1.47)	19.17 (0.98)	18.50 (2.06)
Creatinine (mg/dL)	0.73 (0.04)	0.67 (0.03)	0.65 (0.05)	0.73 (0.04)	0.77 (0.05)	0.78[a] (0.06)	0.70 (0.04)	0.85[a] (0.06)	0.80[a] (0.07)
Potassium (mEq/L)	4.64 (0.11)	4.45 (0.14)	4.33 (0.09)	4.45 (0.20)	4.29 (0.12)	4.10 (0.11)	4.52 (0.09)	4.23 (0.10)	4.07 (0.13)
Chloride (mEq/L)	109.67 (0.43)	110.60 (0.62)	110.13 (0.38)	111.70 (0.96)	108.57[a,d,c] (0.78)	106.83[a,d,c] (0.50)	110.32 (0.46)	104.07[a,d,c] (0.76)	104.97[a,d,c] (0.65)
Sodium (mEq/L)	146.17 (0.48)	146.83 (0.83)	146.00 (0.52)	147.17 (1.01)	147.50 (0.76)	146.67 (0.80)	146.17 (0.75)	145.83 (0.87)	146.00 (0.82)

Ang: angiotensin.
[a] Value is significantly different when compared to the placebo group on this day.
[b] Value is significantly different when compared to day 1.
[c] Value is significantly different when compared to day −1.
[d] Value is significantly different on this day between the furosemide and torsemide groups.

(after acid hydrolysis to aldosterone). In this study, a normal UAldo:C was defined as <1.7 μg/g (the mean + two standard deviations of UAldo:C in a population of 31 normal dogs)[k].

Statistical analysis

A repeated measures analysis was performed for each response variable separately using SAS Proc Mixed. Specifically, period, treatment, day and treatment*day interaction were included as fixed effects. To account for the crossover design, dog and dog*treatment were included as random effects. Bonferonni adjusted *p*-values for treatment main effect and treatment*day interaction were considered separately for 12 serum variables and 13 urine variables. Serum values below the lower limit of quantification (Ang I-5, Ang I-7, Ang II (1-8), Ang III (2-8), Ang IV (3-8), and serum aldosterone) were replaced by a value of half of the lower limit of quantification for purposes of analysis. Only those serum and urine variables with Bonferonni adjusted *p*-values <0.05 were investigated with further pairwise comparisons. For each study day, comparisons between treatments were performed using Tukey's method. For each treatment, comparisons of later time points versus initial measurement (either −1, 1, or 2 depending on variable) were performed using Dunnett's method. Residual diagnostic plots were used to evaluate model assumptions. To satisfy the assumption of normality, all blood variables, fractional excretion of Cl^-, and UAldo:C were analyzed after log transformed. Correlation between variables 24-h UAldo:C and 'spot' UAldo:C was calculated accounting for repeated measures on subjects [23]. The correlation was calculated on the original and

[k] Ames, MK, unpublished data.

Table 3 Urine analytes in healthy dogs treated with placebo, furosemide, or torsemide on treatment days 2, 6, and 10. Values are listed as mean ± standard error of mean (mean [SEM]).

Urine parameter	Placebo			Furosemide			Torsemide		
	Day 2	Day 6	Day 10	Day 2	Day 6	Day 10	Day 2	Day 6	Day 10
FE Cl^- (%)	0.57 (0.17)	0.45 (0.08)	1.23 (0.88)	1.22 (0.15)	0.42 (0.17)	0.58 (0.21)	1.32 (0.18)	0.42 (0.08)	0.43 (0.11)
FE Na^+ (%)	0.27 (0.06)	0.20 (0.05)	0.18 (0.03)	0.65 (0.11)	0.28 (0.10)	0.37 (0.14)	0.73 (0.12)	0.28 (0.04)	0.32 (0.09)
FE K^+ (%)	13.58 (2.49)	12.2 (1.27)	11.08 (2.11)	15.28 (1.02)	12.73 (1.55)	14.63 (1.82)	15.03 (1.65)	15.45 (1.40)	14.20 (1.06)
Na^+/K^+ ratio	0.61 (0.06)	0.54 (0.11)	0.62 (0.08)	1.32[a] (0.15)	0.65[c] (0.24)	0.81[c] (0.25)	1.47[a] (0.13)	0.60[c] (0.06)	0.71[c] (0.16)
24-h water consumption (mL)	515 (93.30)	560.0 (157.40)	492[a] (173.13)	681.50 (142.68)	781.50 (151.21)	1,080[a,d] (59.63)	641.33 (121.18)	760.67 (153.66)	1,097.75[a,d] (209.46)
Total urine volume (mL)	256 (70.53)	252 (67.78)	300.17 (74.82)	437[a] (23.76)	345 (28.99)	443.83 (78.16)	476.67[a] (54.40)	467.83[a] (46.25)	625.67[a,b,c] (92.65)
24-h UAldo:C (ug/g)	0.79 (0.04)	0.95 (0.19)	0.81 (0.14)	0.77 (0.13)	3.36[a,c] (0.79)	2.88[a,c] (0.58)	0.73 (0.15)	3.43[a,c] (0.60)	2.59[a,c] (0.57)
UCl^- (mmol)	33.61 (11.94)	29.10 (7.12)	31.53 (8.30)	63.03[a] (4.33)	16.89[c] (4.03)	23.09[c] (5.78)	66.33[a] (9.78)	20.80[c] (7.42)	24.04[c] (9.40)
UNa^+ (mmol)	19.90 (6.10)	17.37 (4.20)	19.15 (4.17)	44.32[a] (3.30)	14.14 (3.98)	18.88 (4.79)	46.85[a] (5.17)	17.81 (5.32)	20.77 (5.92)
UK^+ (mmol)	31.79 (7.70)	32.46 (6.68)	33.31 (7.55)	35.03 (2.96)	24.15 (3.76)	25.93 (4.62)	33.76 (5.65)	28.60 (6.02)	29.94 (5.65)
USG	1.038 (0)	1.037 (0)	1.033 (0)	1.017 (0)	1.020 (0)	1.016 (0)	1.014 (0)	1.015 (0)	1.014 (0)

[a] Value is significantly greater on this day when compared to placebo group.
[b] Value is significantly greater than the furosemide group on this day.
[c] Value is significantly different when compared to day 2.
[d] Data point was obtained from only 4/6 dogs. FE: fractional excretion; h: hour; USG: urine specific gravity.

log-transformed scales. Analyses were carried out using a commercially available statistical software[l].

Results

At day −1, there were no differences between any group in serum BUN, creatinine, Na^+, K^+, or Cl^- concentrations. Similarly, the urine specific gravity was not different between groups on day −1. Throughout the treatment periods, there were no significant differences in body weight, blood pressure, heart rate, BUN, Na^+, or K^+ between treatment groups, or within treatment groups over time. The blood pressure, heart rate, BUN, and creatinine remained within reference intervals in all dogs throughout the study. Blood pressure, body weight, and heart rate values are outlined for each treatment and sampling day in Table 1. Mild hypochloremia was observed in both diuretic groups. The average serum Cl^- concentrations on days 5 and 9 were significantly lower in the furosemide ($p=0.03$ and $p<0.001$, respectively) and torsemide ($p<0.001$) groups, as compared to the placebo group. Within both diuretic groups, the serum Cl^- concentrations were significantly lower on days 5 ($p<0.001$) and 9 ($p<0.001$) when compared to day −1. The average serum Cl^- concentration was lower in the torsemide group when compared to the furosemide group on days 5 ($p<0.001$) and 9 ($p=0.04$). The average serum creatinine concentration in the furosemide group was significantly greater than the placebo group on day 9 ($p=0.04$). The average serum creatinine concentration in the torsemide group was significantly greater than the placebo group on days 5 ($p=0.006$) and 9 ($p=0.02$). Serum values are outlined for each treatment and sampling day in Table 2.

When the two diuretic groups were compared, there were no differences in the 24-h UAldo:C, spot-UAldo:C or serum equilibrium concentrations of aldosterone, AngI, AngII, Ang(1,7), Ang(1,5), AngIII, or AngIV (see Table 3). RAAS activation is demonstrated in Fig. 1. The 24-h UAldo:C ($p<0.001$), and serum equilibrium concentrations of aldosterone ($p<0.005$), AngI ($p<0.001$), AngII ($p<0.001$), Ang(1,7) ($p<0.001$), Ang(1,5) ($p<0.001$), and AngIV ($p<0.007$) were significantly greater in the diuretic groups when compared to the placebo group on days 5/6 and 9/10. These components also increased significantly over the study period in both diuretic

[l] SAS, version 9.4, SAS Institute Inc, Cary, North Carolina, USA.

groups. Serum equilibrium AngIII concentrations in the diuretic groups did not differ significantly from placebo. The 24-h and spot UAldo:C for each dog were significantly correlated ($p<0.0001$).

Daily urine volumes and a graphical representation of the diuretic effect of each drug (24-h urine volumes) are shown in Fig. 2. The 24-h urine volumes (mL/kg/day) in the diuretic groups were significantly greater than that in the placebo group on day 2 ($p=0.03$) (furosemide) and days 2 ($p=0.01$), 6 ($p=0.01$), and 10 ($p<0.001$) (torsemide). The 24-h urine volume in the torsemide group was significantly greater than that in the furosemide group on day 10 ($p=0.03$). The only change in urine volume over time within a treatment group occurred in the torsemide group, where the daily urine volume on day 10 exceeded that of day 2 ($p=0.03$). Water intake did not differ significantly between diuretic groups on any day.

The total urinary excretion of Na^+ and Cl^- (mmol/kg/day) did not differ between the furosemide and torsemide groups on any day (Fig. 3). The total urinary excretion of Na^+ and Cl^- was greater in the diuretic groups when compared to that in the placebo group only on day 2 ($p<0.005$). The total urinary excretion of Na^+ and Cl^- decreased significantly in the diuretic groups on days 6 and 10 when compared to day 2 ($p<0.001$). There was no difference in the urinary excretion of K^+ between treatment groups, or within treatment groups over time. The urinary Na^+/K^+ initially increased, with the diuretic groups being significantly greater than the placebo on day 2 ($p<0.001$). On the following sampling days (days 6 and 10), the urinary Na^+/K^+ decreased and was no longer significantly different than placebo. The EFWC did not differ significantly between treatment groups on any day (Fig. 2), though it increased significantly between days 2 and 10 in dogs receiving diuretic ($p=0.006$, furosemide; $p=0.01$, torsemide). Urine values are outlined for each treatment and sampling day in Table 3.

Discussion

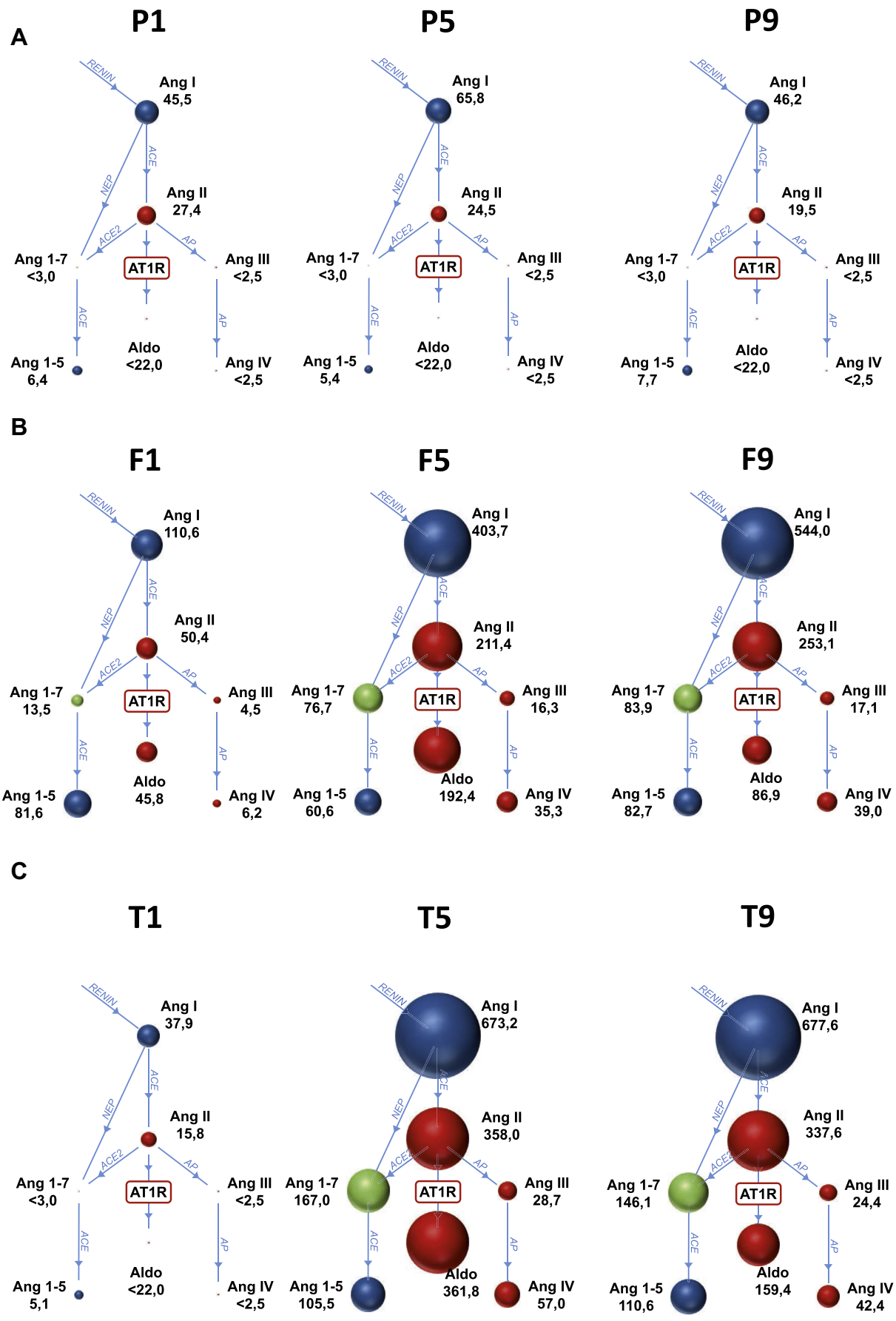
At approximately one-twentieth the dosage, torsemide caused a similar diuresis to furosemide during the 10-day study period. Both diuretics led to a significant increase in the global RAAS activity, as evidenced by significant increases in the UAldo:C and serum aldosterone concentrations and serum equilibrium angiotensin peptide concentrations. At approximately equipotent dosages, the degree of RAAS activation did not differ significantly between the two diuretics. There was also no significant difference in the magnitude of kaliuresis between any of the groups, decreasing support for an MR antagonizing effect of torsemide at this dosage.

Diuretic-induced activation of RAAS was evidenced by significantly greater serum AngI, AngII, Ang(1,7), Ang(1,5), and AngIV, aldosterone, and UAldo:C in the diuretic groups when compared to placebo on study days 5/6 and 9/10 (Fig. 1). The metabolism/degradation of AngI and AngII is of interest, as these pathways reflect their breakdown and result in peptides that are 'active' and, in some cases, counter-regulatory to RAAS activation. The counter-regulatory pathway involving Ang(1,7) (the angiotensin-converting enzyme 2-Ang(1,7)-Mas receptor) axis is currently being investigated, as it appears to elicit protective actions, including increased nitric oxide synthesis and vasodilation [24]. Ang(1,7) has been shown to be the dominant metabolite of AngII in several tissues [25,26], which appears to be the case in these dogs with diuretic-induced activation of their RAAS. Angiotensin II is also metabolized by aminopeptidase A to form AngIII, which like its parent peptide, is capable of binding both the AngII type-1 and -2 receptors [27]. Angiotensin III can then be metabolized to AngIV via aminopeptidase N. Both of these angiotensin peptides lead to increased atrial stretch-induced atrial natriuretic peptide secretion in animal models, via the AngII type-2 receptor and the insulin-regulated aminopeptidase, respectively [28,29].

The significant correlation between the 24-h and 'spot' UAldo:C ($p<0.0001$) is similar to the findings of a previous study where 'spot' urine samples were significantly correlated with 24-h urine aldosterone excretion [30]. Although correlation was not evaluated between UAldo:C and serum aldosterone concentrations, in the 31 samples with a serum aldosterone below the limit of detection of the LC-MS/MS assay (<22 pmol/L), 30 samples (97%) had a normal (<1.7 μg/g) 24-h UAldo:C and 31 samples (100%) had a normal UAldo:C spot value. A normal spot UAldo:C is therefore supportive of normal circulating aldosterone levels.

In dogs, torsemide has historically been considered to be about 10 times more potent (based on urine production per milligram of the drug) than furosemide. Closer inspection of urine production in early studies [4,5] and a recent abstract[d] support that this factor is, on average, greater than 10. A recent monograph[m] shows an increasing relative

[m] Jacobs M. 2016. Population pharmacokinetics and pharmacodynamics study of torasemide and furosemide after oral repeated administrations of torasemide or furosemide in dogs. ISEMID® Marketing Authorisation dossier. Data on file. Ceva Santé Animale.


A
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RENIN
Ang I
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ACE
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27,4
ACE2
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Ang 1-7
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Ang III
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Aldo
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Ang IV
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RENIN
Ang I
65,8
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24,5
ACE2
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Ang 1-5
60,6
Aldo
192,4
Ang IV
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ACE
NEP
Ang II
253,1
ACE2
AP
Ang 1-7
83,9
AT1R
Ang III
17,1
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Ang 1-5
82,7
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Ang IV
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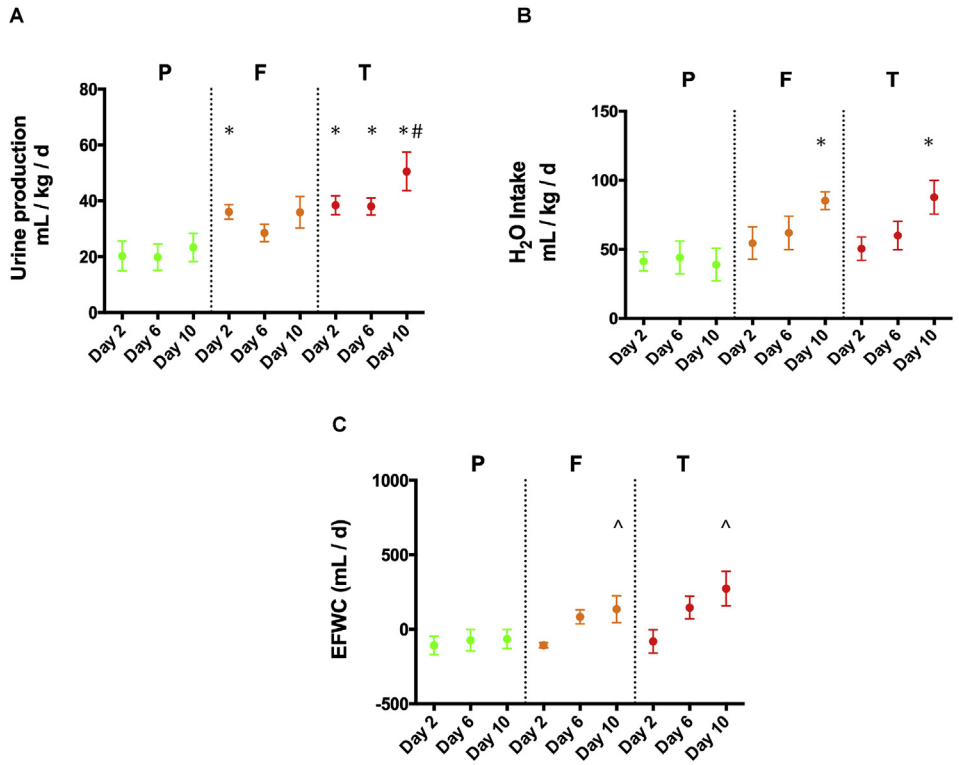

Fig. 2 Mean (standard area of mean) for urine production (A), water intake (B), and electrolyte free water clearance (C) shown for all treatments on study days 2, 6, and 10. *Value is significantly greater on this day when compared to placebo group. #Value is significantly greater than the furosemide group on this day. ^Value is significantly greater than day 2. EFWC: electrolyte free water clearance; F: furosemide; H_2O: water; P: placebo; T: torsemide.

potency (from approximately 10—20 times) as the dosage of torsemide increases. In that monograph, when torsemide was dosed at 0.2 mg/kg once daily, it was about 10 times more potent than furosemide. The results of our study support that, when torsemide is given at a dosage of 0.1 mg/kg q 12 h, the potency factor is closer to 20×, implying that the frequency of dosing affects this potency relationship. In fact, on day 10 of this study, torsemide, at the one-twentieth dosage of furosemide, led to a significantly greater urine production than furosemide. The increase in urine production between days 6 and 10 in the torsemide group suggests that diuresis might not have yet 'plateaued' at study end.

These data suggest that reevaluating patients only once at 7—10 days after transition to (or the initiation of) torsemide may not allow for a full evaluation of diuretic effect (and impact on the patient's serum renal values and serum electrolytes). In this short-term study, torsemide caused more chloride loss than furosemide, whereas serum sodium and potassium concentrations did not differ significantly among groups. However, we observed a decrease in aldosterone levels between day 5 and day 9 within both groups of diuretic treatment, which might be linked to a trend in the reduction of serum potassium levels. Chronic monitoring of renal values and serum electrolytes during diuretic therapy is, of course, important. In the recent TEST trial [10], dogs with myxomatous mitral valve disease and congestive heart failure receiving torsemide had significantly higher creatinine values and significantly lower potassium values at study end (day 84) when compared to dogs receiving furosemide.

Fig. 1 Serum equilibrium concentrations of angiotensin peptides (renin-angiotensin system [RAS]-Fingerprint®) and serum aldosterone concentrations from dogs receiving placebo (A), furosemide (B), and torsemide (C) on study days 1, 5, and 9. The RAS-Fingerprint® is illustrated as a pedigree starting at angiotensin I. Each intersection represents a specific peptide fragment symbolized by colored spheres; enzymes involved in the reactions are annotated on connecting lines. Size of spheres and numbers beside them represent the mean serum concentrations of angiotensins of the six dogs in picomoles per liter (pmol/L). (1-5): angiotensin(1-5); (1-7): angiotensin(1-7); ACE: angiotensin-converting enzyme; ACE2: angiotensin-converting enzyme 2; AngI: angiotensin I; AngII: Angiotensin II; AngIII: Angiotensin III; AngIV: Angiotensin IV; Aldo: aldosterone; AP: aminopeptidase; AT_1R: Angiotensin II Type-1 Receptor; F: furosemide; NEP: neutral endopeptidase; P: placebo; T: torsemide.

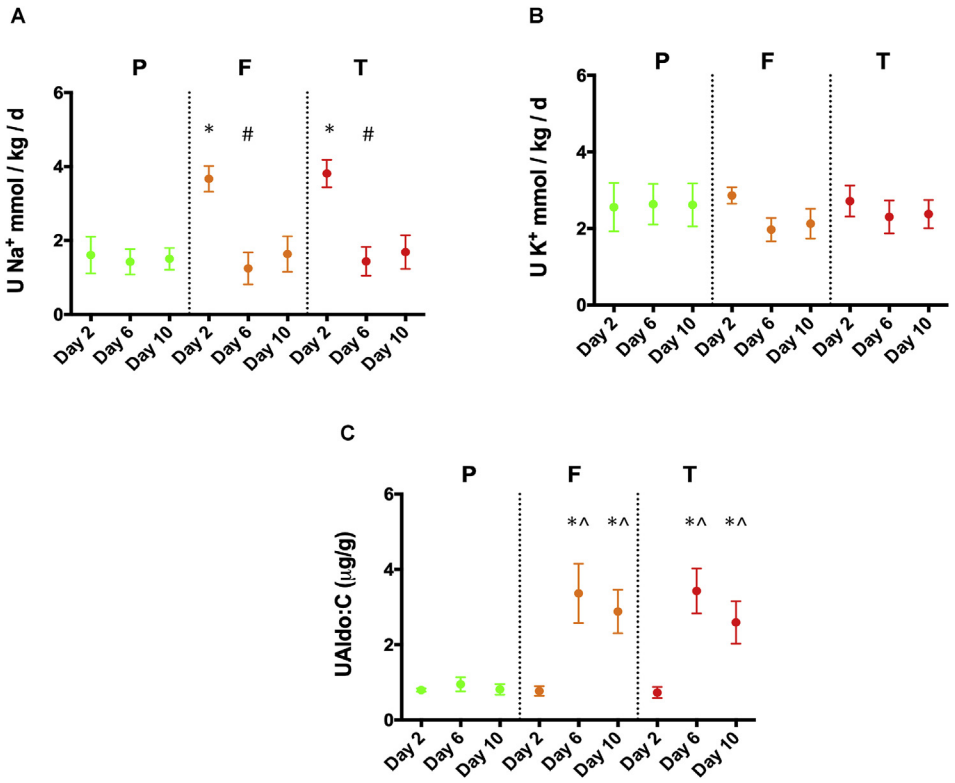

Fig. 3 Mean (standard area of mean) for 24-h urine sodium excretion (A), 24-h urine potassium excretion (B), and the 24-h urine aldosterone to creatinine ratio (C) shown for all treatments on study days 2, 6, and 10. *Value is significantly greater on this day when compared to placebo group. #Value is significantly lower than day 2. ^Value is significantly greater than day 2. F: furosemide; P: placebo; T: torsemide; UAldo:C: urine aldosterone to creatinine ratio; UK^+: 24-h urine potassium excretion, UNa^+: 24-h urine sodium excretion.

The increased 24-h Na^+ and Cl^- excretion and low (negative) EFWC on day 2 in the diuretic groups reflect a solute rich urine in response to the first two dosages of diuretics. This is appropriate for healthy, hydrated dogs with presumably normal renal interstitial hypertonicity. The subsequent increase in EFWC in both diuretic groups reflects a continued diuresis, yet with increased free water excretion. The significant decline in both Na^+ and Cl^- excretion on days 6 and 10 was due, at least in part, to activation of RAAS and increased sodium reabsorption. Despite this, there is continued diuresis and production of hypotonic urine (increase in EFWC). This likely occurs because of a loss of the renal interstitial hypertonicity and loss of the osmotic gradient and reduction in water reabsorption in the distal nephron after diuresis. Because the thick ascending loop of Henle has relatively low water permeability, loop diuretic-induced blockade of the $Na^+/K^+/2Cl^-$ cotransporter impedes the recreation of a hypertonic interstitium. Although there may be concurrent non-osmotic stimulation of vasopressin release, the reduction in the osmotic gradient reduces the efficacy of a vasopressin-induced increase in epithelial Na^+ channels, preventing increased water permeability in the distal tubules. Finally, water intake in both diuretic groups was significantly greater than the placebo on day 10.

It has been postulated that torsemide may antagonize the MR in the kidney [31]. These data reported here, however, are more supportive of MR activation at the torsemide dosage of 0.1 mg/kg PO q 12 h, as MR antagonism in the kidney would be expected to cause an increase in the urinary Na^+/K^+ [32]. It is possible that torsemide may antagonize the MR in the kidney at higher dosages. Torsemide's effect on the MR may also vary by tissue, as one recent study demonstrated that torsemide does not bind the MR in rat cardiomyocytes [33]. As anti-aldosterone and anti-fibrotic effects of torsemide are postulated, further study is clearly needed [34]. Also, evaluation of an MR antagonizing effect should be explored further in dogs with chronic heart failure and excess aldosterone secretion and increased activation of the epithelial sodium channels and Na^+/K^+ ATPase of the distal convoluted tubules.

The results of this study must be interpreted in light of several limitations. This study was

performed in normal dogs and does not replicate the neurohormonal, cardiovascular, and renal derangements that attend the syndrome of heart failure. Also, the subacute administration of these diuretics does not replicate the chronic pharmacotherapy of dogs with heart failure. The full diuretic effect of torsemide also may not have been realized in this 10-day study. Additionally, in dogs with naturally occurring heart disease, the magnitude and 'phenotype' of RAAS peptide activation and diuretic response are likely variable between individuals and within an individual over time. Another limitation is the small number of dogs and the use of a cross over design. Precautions taken to minimize the effects of the crossover design included randomization of the dogs' treatment order, an appropriate washout period, and the use of a statistical model that accounts for period effects. Due to the study design where each of the six possible sequences of treatments was assigned to exactly one subject, it was not possible to evaluate sequence effects. The use of a washout period containing 23 elimination half-lives, however, should have minimized the risk for sequence effect on response variables.

Conclusions

This study more comprehensively demonstrates the previously known diuretic-induced activation of RAAS using a novel method of RAAS quantification. A similar diuresis was obtained when torsemide was administered to healthy dogs at the one-twentieth dosage of furosemide. At the dosages studied here, the degree of RAAS activation did not differ significantly between furosemide and torsemide. Also, the potassium excretion profile after furosemide and torsemide administration supports increased aldosterone production and MR activation and these data do not support an MR antagonizing effect of torsemide in the kidney at this dosage and during subacute administration to healthy dogs.

Conflict of Interest Statement

This study was funded by both the Colorado State University College of Veterinary Medicine and Biomedical Sciences Research Council and Ceva Sante Animale.

Dr. Marisa Ames is a consultant for Ceva Sante Animale.

Dr. Marko Poglitsch is the CEO of Attoquant Diagnostics GmbH.

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References

[1] Bikdeli B, Strait KM, Dharmarajan K, Partovian C, Coca SG, Kim N, Li SX, Testani JM, Khan U, Krumholz HM. Dominance of furosemide for loop diuretic therapy in heart failure: time to revisit the alternatives? J Am Coll Cardiol 2013;61:1549—50.

[2] Ghys A, Denef J, Delarge J, Georges A. Renal effects of the high ceiling diuretic torasemide in rats and dogs. Arzneimittelforschung 1985;35:1527—31.

[3] Sogame Y, Okano K, Hayashi K, Uchida T, Tsuda Y. Urinary excretion profile of torasemide and its diuretic action in dogs. J Pharm Pharmacol 1996;48:375—9.

[4] Uechi M, Matsuoka M, Kuwajima E, Kaneko T, Yamashita K, Fukushima U, Ishikawa Y. The effects of the loop diuretics furosemide and torasemide on diuresis in dogs and cats. J Vet Med Sci 2003;65:1057—61.

[5] Hori Y, Takusagawa F, Ikadai H, Uechi M, Hoshi F, Higuchi S. Effects of oral administration of furosemide and torsemide in healthy dogs. Am J Vet Res 2007;68:1058—63.

[6] Cosin J, Diez J, investigators T. Torasemide in chronic heart failure: results of the TORIC study. Eur J Heart Fail 2002;4:507—13.

[7] Mentz RJ, Hasselblad V, DeVore AD, Metra M, Voors AA, Armstrong PW, Ezekowitz JA, Tang WH, Schulte PJ, Anstrom KJ, Hernandez AF, Velazquez EJ, O'Connor CM. Torsemide versus furosemide in patients with acute heart failure (from the ASCEND-HF trial). Am J Cardiol 2016;117:404—11.

[8] Oyama MA, Peddle GD, Reynolds CA, Singletary GE. Use of the loop diuretic torsemide in three dogs with advanced heart failure. J Vet Cardiol 2011;13:287—92.

[9] Peddle GD, Singletary GE, Reynolds CA, Trafny DJ, Machen MC, Oyama MA. Effect of torsemide and furosemide on clinical, laboratory, radiographic and quality of life variables in dogs with heart failure secondary to mitral valve disease. J Vet Cardiol 2012;14:253—9.

[10] Chetboul V, Pouchelon JL, Menard J, Blanc J, Desquilbet L, Petit A, Rougier S, Lucats L, Woehrle F, investigators Ts. Short-term efficacy and safety of torasemide and furosemide in 366 dogs with degenerative mitral valve disease: the TEST study. J Vet Intern Med 2017;31:1629—42.

[11] Bayliss J, Norell M, Canepa-Anson R, Sutton G, Poole-Wilson P. Untreated heart failure: clinical and neuroendocrine effects of introducing diuretics. Br Heart J 1987;57:17—22.

[12] Francis GS, Benedict C, Johnstone DE, Kirlin PC, Nicklas J, Liang CS, Kubo SH, Rudin-Toretsky E, Yusuf S. Comparison of neuroendocrine activation in patients with left ventricular dysfunction with and without congestive heart failure. A substudy of the Studies of Left Ventricular Dysfunction (SOLVD). Circulation 1990;82:1724—9.

[13] Caro-Vadillo A, Ynaraja-Ramirez E, Montoya-Alonso JA. Effect of torsemide on serum and urine electrolyte levels in dogs with congestive heart failure. Vet Rec 2007;160:847—8.

[14] Ames MK, Atkins CE, Eriksson A, Hess AM. Aldosterone breakthrough in dogs with naturally occurring myxomatous mitral valve disease. J Vet Cardiol 2017;19:218—27.

[15] Ames MK, Atkins CE, Lantis AC, Werre SR. Effect of furosemide and high-dosage pimobendan administration on the renin-angiotensin-aldosterone system in dogs. Am J Vet Res 2013;74:1084—90.
[16] Ames MK, Atkins CE, Lee S, Lantis AC, zumBrunnen JR. Effects of high doses of enalapril and benazepril on the pharmacologically activated renin-angiotensin-aldosterone system in clinically normal dogs. Am J Vet Res 2015;76:1041—50.
[17] Atkins CE, Rausch WP, Gardner SY, Defrancesco TC, Keene BW, Levine JF. The effect of amlodipine and the combination of amlodipine and enalapril on the renin-angiotensin-aldosterone system in the dog. J Vet Pharmacol Ther 2007;30:394—400.
[18] Lantis AC, Ames MK, Atkins CE, DeFrancesco TC, Keene BW, Werre SR. Aldosterone breakthrough with benazepril in furosemide-activated renin-angiotensin-aldosterone system in normal dogs. J Vet Pharmacol Ther 2015;38:65—73.
[19] Lantis AC, Ames MK, Werre S, Atkins CE. The effect of enalapril on furosemide-activated renin-angiotensin-aldosterone system in healthy dogs. J Vet Pharmacol Ther 2015;38:513—7.
[20] Lantis AC, Atkins CE, DeFrancesco TC, Keene BW, Werre SR. Effects of furosemide and the combination of furosemide and the labeled dosage of pimobendan on the circulating renin-angiotensin-aldosterone system in clinically normal dogs. Am J Vet Res 2011;72:1646—51.
[21] Paulin A, Schneider M, Dron F, Woehrle F. A pharmacokinetic/pharmacodynamic model capturing the time course of torasemide-induced diuresis in the dog. J Vet Pharmacol Ther 2016;39:547—59.
[22] Jeunesse E, Woehrle F, Schneider M, Lefebvre HP. Effect of spironolactone on diuresis and urine sodium and potassium excretion in healthy dogs. J Vet Cardiol 2007;9:63—8.
[23] Bland JM, Altman DG. Calculating correlation coefficients with repeated observations: Part 1-Correlation within subjects. BMJ 1995;310:446.
[24] Bader M. Tissue renin-angiotensin-aldosterone systems: targets for pharmacological therapy. Annu Rev Pharmacol Toxicol 2010;50:439—65.
[25] Cui L, Nithipatikom K, Campbell WB. Simultaneous analysis of angiotensin peptides by LC-MS and LC-MS/MS: metabolism by bovine adrenal endothelial cells. Anal Biochem 2007;369:27—33.
[26] Velez JC, Ierardi JL, Bland AM, Morinelli TA, Arthur JM, Raymond JR, Janech MG. Enzymatic processing of angiotensin peptides by human glomerular endothelial cells. Am J Physiol Renal Physiol 2012;302:F1583—94.
[27] Park BM, Gao S, Cha SA, Park BH, Kim SH. Cardioprotective effects of angiotensin III against ischemic injury via the AT2 receptor and KATP channels. Phys Rep 2013;1:e00151.
[28] Park BM, Cha SA, Han BR, Kim SH. Angiotensin IV stimulates high atrial stretch-induced ANP secretion via insulin regulated aminopeptidase. Peptides 2015;63:30—7.
[29] Park BM, Oh YB, Gao S, Cha SA, Kang KP, Kim SH. Angiotensin III stimulates high stretch-induced ANP secretion via angiotensin type 2 receptor. Peptides 2013;42:131—7.
[30] Gardner SY, Atkins CE, Rausch WP, DeFrancesco TC, Chandler DW, Keene BW. Estimation of 24-h aldosterone secretion in the dog using the urine aldosterone:creatinine ratio. J Vet Cardiol 2007;9:1—7.
[31] Uchida T, Yamanaga K, Nishikawa M, Ohtaki Y, Kido H, Watanabe M. Anti-aldosteronergic effect of torasemide. Eur J Pharmacol 1991;205:145—50.
[32] Eudy RJ, Sahasrabudhe V, Sweeney K, Tugnait M, King-Ahmad A, Near K, Loria P, Banker ME, Piotrowski DW, Boustany-Kari CM. The use of plasma aldosterone and urinary sodium to potassium ratio as translatable quantitative biomarkers of mineralocorticoid receptor antagonism. J Transl Med 2011;9:180.
[33] Gravez B, Tarjus A, Jimenez-Canino R, El Moghrabi S, Messaoudi S, Alvarez de la Rosa D, Jaisser F. The diuretic torasemide does not prevent aldosterone-mediated mineralocorticoid receptor activation in cardiomyocytes. PLoS One 2013;8:e73737.
[34] Adam O, Zimmer C, Hanke N, Hartmann RW, Klemmer B, Bohm M, Laufs U. Inhibition of aldosterone synthase (CYP11B2) by torasemide prevents atrial fibrosis and atrial fibrillation in mice. J Mol Cell Cardiol 2015;85:140—50.