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Evaluation of subacute change in RAAS activity (as indicated by urinary aldosterone:creatinine, after pharmacologic provocation) and the response to ACE inhibition

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Abstract

Objective: The objective of this study was to evaluate subacute changes in renin–angiotensin–aldosterone system (RAAS) activity during angiotensin-converting enzyme inhibitor (ACEI) therapy in dogs with experimental RAAS activation.

Methods: Analysis of data (urine aldosterone:creatinine ratio (UAldo:C) and serum angiotensin-converting enzyme activity), in 31 healthy dogs with furosemide or amlodipine-activated RAAS that received an ACEI. When furosemide or amlodipine activation of RAAS preceded ACEI administration, incomplete RAAS blockade (IRB) was defined as a UAldo:C greater than (a) the dog's 'activated' baseline value or (b) a population-derived cut-off value (mean + 2 SD (>1.0 µg/g) of pretreatment UAldo:C from our population of research dogs). In studies where RAAS activation occurred concurrently with ACEIs, IRB was defined as (a) a UAldo:C greater than either twofold the dog's prestimulation baseline value or (b) 1.0 µg/g. Dogs were followed for 7–17 days.

Results: Serum angiotensin-converting enzyme activity was measured in 19 dogs and was significantly reduced ($P<0.0001$) after ACEI administration. The overall incidence of IRB, when RAAS activation preceded ACEI administration, was 33% and 8% for definitions (a) and (b), respectively. The overall incidence of IRB, when ACEIs were concurrent with RAAS activation, was 65% and 61% for definitions (a) and (b), respectively.

Conclusion: Increases in UAldo:C, despite ACEI administration, is evidence of IRB in this subacute model of experimental RAAS activation and suppression.

Keywords

Aldosterone breakthrough, RAAS activation, angiotensin-converting enzyme inhibitors, mineralocorticoid receptor blockers, heart failure

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Urine aldosterone to creatinine ratio

Chronic activation of the renin–angiotensin–aldosterone system (RAAS) is a major consequence and perpetuator of diminished cardiac performance in patients with cardiovascular and renal disease, leading to fluid retention, vascular and myocardial remodeling, endothelial and baroreceptor dysfunction.[1–11] The consequences of chronic and excessive aldosterone secretion on cardiovascular and renal tissues include fibrosis, inflammation, endothelial dysfunction and secondary myocardial hypertrophy. These

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adverse effects have been demonstrated in animal models of both cardiovascular and renal disease.[4,6,12–14] The interruption of RAAS is therefore a key therapeutic strategy in the therapy of heart disease, hypertension and proteinuric renal disease, and is achieved by the administration of angiotensin-converting-enzyme inhibitors (ACEIs), angiotensin II receptor blockers (ARBs) and mineralocorticoid receptor blockers, alone or in combination. In the dog, myxomatous mitral valve disease and primary dilated cardiomyopathy are the most common acquired heart diseases and the most important cause of cardiac morbidity and mortality in this species. Multiple controlled clinical trials in dogs with heart failure due to both myxomatous mitral valve disease and dilated cardiomyopathy have demonstrated the benefits of ACEIs and this drug class is considered 'standard therapy' of heart failure due to these diseases.[15–19]

The pharmacologic interruption of RAAS is studied in the authors' laboratory by a model of RAAS activation, using either furosemide or amlodipine.[20–26, a] This model is very useful in that the drugs used to mimic RAAS activation seen in heart failure are two of the very drugs used in the management of heart failure in humans and in dogs. RAAS activation is evaluated by the measurement of the urine aldosterone:creatinine ratio (UAldo:C), using a previously validated radioimmunoassay.[b,c,d] The UAldo:C, using spot urine samples and a radioimmunoassay methodology, has been correlated with 24-hour urine aldosterone excretion in the dog.[27] This assay measures both free aldosterone and one of its more abundant metabolites, aldosterone 18β-glucuronide. The administration of furosemide (2 mg/kg by mouth (po) q12 h) or amlodipine (0.5 mg/kg po q12 h) leads to a significant increase in the UAldo:C, indicating RAAS activation.[20–26, a] This activation appears to plateau by the fifth day of drug administration.[22] The activation of RAAS in this model is very consistent and eliminates the minute-to-minute variation seen with blood aldosterone concentrations, particularly when two samples are collected, one in the morning and one in the evening and equal aliquots of each are combined for analysis. Further validation of the UAldo:C, as a measure of RAAS activation, comes in the expected response of the UAldo:C to perturbations, such as volume reduction, blood pressure reduction, reduced sodium intake and stress.

Administration of either benazepril (0.5 mg/kg po q12h) or enalapril (0.5 mg/kg po q12 h) to healthy laboratory dogs with amlodipine or furosemide-induced activation of RAAS leads to a significant reduction in (circulating) angiotensin-converting enzyme (ACE) activity.[24,26] In a subset of these dogs, the UAldo:C values exceed those at baseline (sometimes by twofold), or are greater than those of normal dogs, despite significant suppression of circulating ACE activity. These dogs, therefore, have incomplete RAAS blockade (IRB). Evaluation of RAAS activity on an individual basis in these subacute experiments has not been performed. To the authors' knowledge, the UAldo:C is little used in human patients and may prove a useful index of RAAS activation aiding in determining the individual need for more aggressive RAAS suppression.

The UAldo:C assay may also be useful in the study of the phenomenon of aldosterone breakthrough (ABT). ABT refers to incomplete pharmacologic blockade of the RAAS by ACEIs and/or ARBs. The existence of ABT is well accepted, yet remains poorly understood and definitions vary.[28–32] The clinical definition of ABT is typically based on a single blood aldosterone concentration and is either a baseline definition (a patient's blood aldosterone concentration increases despite ACEI/ARB therapy) or a cut-off definition (while receiving an ACEI and/or ARB, a patient's blood aldosterone concentration is greater than the mean or upper reference interval of a normal population). It may take weeks for the maximal beneficial effect of RAAS antagonists to be realized[33,34] and evaluation for ABT may be best performed after this subacute period. Because there is no consensus regarding a definition of ABT, its estimated incidence is unclear, varying between studies and investigators.[31] In the short-term (<4 weeks) studies presented here, an elevated UAldo:C, despite ACEI administration, is referred to as IRB, rather than ABT, because of the subacute nature of the trials.

The objectives of this study were to analyze individual dog data from published and unpublished studies from the authors' laboratory and to evaluate subacute changes in RAAS activity after both activation and inhibition of RAAS, utilizing our model of amlodipine and furosemide-activated RAAS and the UAldo:C.

Materials and methods

Animals

Five prospective studies were analyzed (Table 1).[20,23,24,26, a,e] Hereafter, they will be referred to by the study number (Table 1). The fifth study had a longitudinal design and the sections are referred to as 5a, 5b and 5c. Each was designed to examine the time course and magnitude of the response of circulating RAAS to the administration of furosemide (2 mg/kg po q12 h) or amlodipine (0.5 mg/kg po q12 h) and drugs that interrupt RAAS, including enalapril (either 0.5 mg/kg po q12 h or 1.0 mg/kg po q12 h) and benazepril (either 1 mg/kg po q24 h or 1.0 mg/kg po q12 h). A total of 50 mature (>1 year of age) dogs were enrolled in the studies. Of these 50 dogs, 31 received an ACEI. These 31 dogs were evaluated for IRB during subacute treatment with an ACEI.

Each dog had been determined to be healthy through evaluation of history, a complete physical examination, and analysis of a database consisting of systemic blood pressure, complete blood count, serum chemistry profile

Table 1. Five subacute studies evaluating pharmacologic RAAS activation as a model of congestive heart failure in normal dogs, with amlodipine and furosemide as RAAS activators.

Studies where ACEI added after RAAS activation	Total dogs receiving an ACEI	Study design	Duration of ACEI (days)	ACE activity measured	IRB criterion 1 >BL (%)	IRB criterion 2 >1.0 µg/g (%)
1. Amlodipine, add Enalapril[20]	6	AMLOD 0.57 mg/kg po q12 h × 10 days Add ENAL 0.57 mg/kg po q12 h days 6–10	4	N	17	0
2. Furosemide, add Benazepril[a]	6	FURO 2 mg/kg po q12 × 17 days Add BNZ 1 mg/kg po q24 h days 10–17	7	N	50	17
3. Furosemide and Benazepril concurrently[23]	5	FURO 2 mg/kg po q12 h And BNZ 1 mg/kg po q24 h × 7 days	7	Y	60	40
4. Furosemide and Enalapril concurrently[24]	8	FURO 2 mg/kg po q12 h And ENAL 0.5 mg/kg po q12 h × 7 days	7	Y	75	75
5a. Furosemide and high-dose Enalapril[26]	6	Experiment 1: FURO 2 mg/kg po q12 h	7	Y	50	67
5b. Furosemide and high-dose Benazepril[26]	Longitudinal study	And ENAL 1 mg/kg po q12 h × 7 days	7	Y	100	100
5c. Amlodipine and high-dose Enalapril[e]		Experiment 2: FURO 2 mg/kg po q12 h And BNZ 1 mg/kg po q12 h × 7 days Experiment 3: AMLOD 0.5 mg/kg po q12 h And ENAL 1 mg/kg po q12 h × 7 days	7	Y	33	17

Only the dogs receiving an ACEI and either amlodipine or furosemide are included. Note that in the column 'Study design', the word 'add' designates studies in which RAAS was activated before adding ACEIs, while the word 'and' indicates that RAAS activation and ACEIs were initiated simultaneously.
RAAS: renin–angiotensin–aldosterone system; ACEI: angiotensin-converting-enzyme-in hibitor; AMLOD: amlodipine; BL: baseline; BNZ: benazepril; ENAL: enalapril; FURO: furosemide; IRB: incomplete RAAS blockade.

and urinalysis. During all studies, the dogs were housed in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care with controlled light/dark cycles. The dogs were fed a standard commercial diet (0.42% sodium and 0.84% chloride on a dry matter basis).[f] Each study was approved by the institutional animal care and use committee at the respective institutions.

Study designs

Studies 1, 2, 3 and 5 were performed at the North Carolina State College of Veterinary Medicine (NCSU CVM). Study 4 was performed at the Virginia-Maryland Regional College of Veterinary Medicine (VA-MD CVM).

Study designs were as follows: Study 1 – six dogs received amlodipine[g] (0.5 mg/kg po q12 h) for 10 days. On day 6, enalapril[h] (0.5 mg/kg po q12 h) was added for the last 4 days of the study. Urine was collected over a 24-hour period on days 0, 5 and 10 and the UAldo:C was measured from these samples. Study 2 – of the 12 dogs in this study, six were included in the evaluation for IRB. These six dogs received furosemide[i] (2 mg/kg po q12 h) for 17 days. On day 10, benazepril[j] (1 mg/kg po q24 h) was added for the last 7 days of the study. Morning and evening urine samples were collected on days 0, 5, 10 and 17 for measurement of the UAldo:C. Study 3 – of the 10 dogs in this study, five were included in the evaluation for IRB. These five dogs received furosemide[i] (2 mg/kg po q12 h) and benazepril[j] (1 mg/kg po q24 h) concurrently for 7

days. Morning and evening urine samples were collected on days –1, –2, 1, 3 and 7 for measurement of the UAldo:C. Study 4 – of the 16 dogs in this study, eight were included in the evaluation for IRB. These dogs received furosemide[i] (2 mg/kg po q12 h) and enalapril[k] (0.5 mg/kg po q12 h) concurrently for 7 days. Morning and evening urine samples were collected on days –1, –2, 1, 4 and 7 for measurement of the UAldo:C. Study 5 – six dogs were included in this longitudinal study consisting of three experiments, which were separated by a 2-week washout period. During the first experiment, all six dogs received furosemide[l,m] (2 mg/kg po q12 h) and enalapril[k] (1 mg/kg po q12 h) concurrently for 7 days. In the second experiment, all six dogs received furosemide[l,m] (2 mg/kg po q12 h) and benazepril[n] (1 mg/kg po q12 h) concurrently for 7 days. In the third experiment, all six dogs received amlodipine[g] (0.5 mg/kg po q12 h) and enalapril[k] (1 mg/kg po q12 h) concurrently for 7 days. For all three experiments, the UAldo:C was measured using equal aliquots of urine, collected in the morning and evening, frozen, and later mixed, from study days –2, –1, 1, 3 and 7.

Serum ACE activity

Serum ACE was evaluated in three studies (studies 3, 4 and 5). In all three studies serum ACE was obtained between 4 and 6 hours after ACEI administration on days –1, –2, 1, 3 and 7,[23,26] days –1, –2, 1, 4 and 7,[24] and days –1, 1, 3 and 7.[26,e] In these three studies, serum was frozen at –70°C and stored prior to measurement of ACE activity by means of a commercially available kit, according to the manufacturer's instructions.

Urine aldosterone:creatinine ratio

Two radioimmunoassays that have been validated in dogs[b,c,d] were used to measure urine aldosterone concentrations over the 11 years of these studies. Urine creatinine was measured by a standard colorimetric assay. For studies 1–4, the UAldo:C was performed at a commercial diagnostic laboratory.[d] For study 5, the UAldo:C was performed using a commercially available radioimmunoassay kit at a veterinary diagnostic laboratory.[b,d]

The methodology for the radioimmunoassays are discussed elsewhere.[20,26] In study 1, urine was collected in metabolic cages at three time points for a 24-hour period. Three millilitres of urine from the combined 24-hour urine sample was frozen at –70°C and submitted for determination of UAldo:C by radioimmunoassay.[b] The protocol for obtaining and analyzing urine was the same in studies 2–5; 5–10 mL of urine were obtained from each dog in the morning and evening for determination of UAldo:C. Samples were refrigerated within 10 minutes of collection and frozen at –70°C within 3 hours of collection. Later, a pooled sample, using equal aliquots from the morning and evening urine samples was thawed, mixed, refrozen and submitted for determination of UAldo:C by radioimmunoassay.[b,c,d] The baseline UAldo:C was determined by averaging the two 'baseline days' (–2 and –1) in studies 2–4. The baseline UAldo:C for the dogs in study 5 was determined by averaging all six of the 'baseline days' in this longitudinal study.

Definition of IRB

The definition of IRB was dependent on whether ACEI administration was initiated after, or concurrently with, pharmacologic RAAS activation. When furosemide or amlodipine activation of RAAS preceded ACEI administration, a dog was defined as having IRB if its final day's UAldo:C was: greater than either (a) its 'activated' baseline or (b) population-derived cut-off value (mean + 2 SD (>1.0 μg/g) of pretreatment UAldo:C from our population of research dogs). In studies in which furosemide or amlodipine activation of RAAS occurred concurrently with ACEI, a dog was defined as having IRB if its final day's UAldo:C was either (a) greater than twofold higher than its prestimulation baseline value or (b) greater than the aforementioned cut-off value of 1.0 μg/g.

Statistical analysis

The incidence of IRB using the criteria is reported for each of the five studies. The incidence of IRB for all three groups in study 5 (dogs receiving high dosages of ACEI) was compared to the incidence of IRB in standard dose studies (1, 3 and 4) using a Fisher's exact test. The incidence of IRB was compared between studies that employed enalapril and benazepril and between studies that employed furosemide and amlodipine as RAAS stimulants with the Fisher's exact test. Comparison of the two different radioimmunoassays used in the studies was carried out by evaluating baseline UAldo:C values from 51 laboratory dogs from the first radioimmunoassay[b] and six laboratory dogs from the second radioimmunoassay[c] and these radioimmunoassays were compared using the Wilcoxon rank sum and the Kolmogorov–Smirnov test. The ages of dogs from the two institutions (NCSU and VA-MD CVMs) were also compared using these two tests. The criteria for statistical significance was set at α=0.05. Analyses were performed using the MIXED, FREQ and NPAR1WAY procedures in SAS/STAT[p] version 9.4.

Results

Dogs

Thirty-one dogs received either furosemide or amlodipine and an ACEI (either enalapril or benazepril) in the five studies. Twenty-three dogs were purpose-bred research dogs from NCSU CVM and eight dogs were purpose-bred research dogs from VA-MD CVM. There were six intact

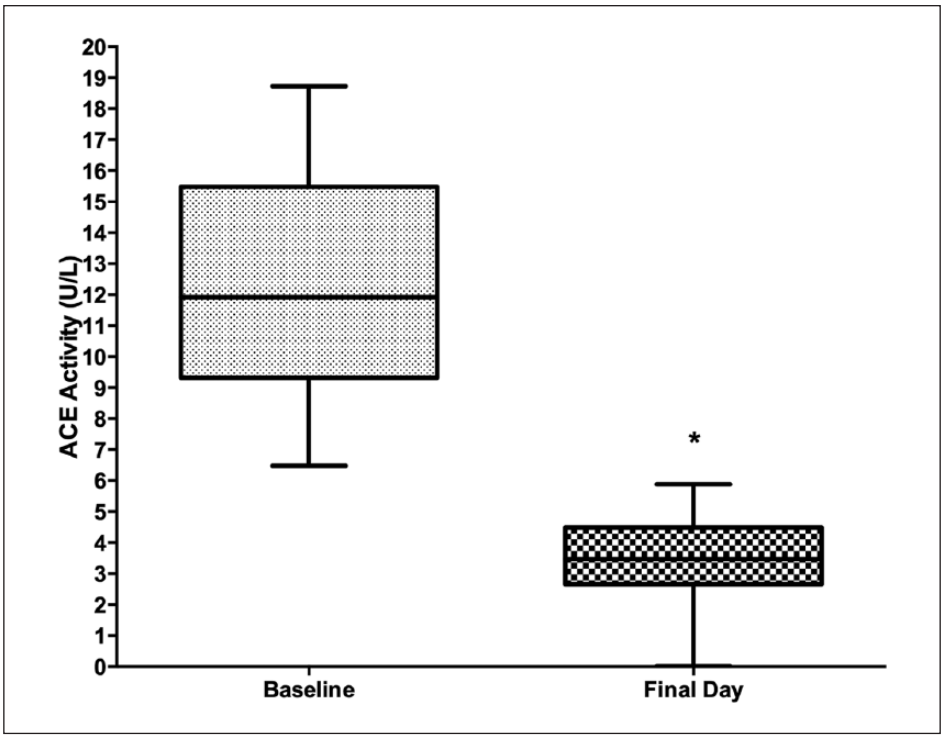

Figure 1. Box and whisker (mean, minimum and maximum) plot of serum ACE activity (U/L) for all dogs in studies 3, 4 and 5 (n=19 dogs) at baseline and on the final study day. The duration of ACEI administration on the final day was 7 days in studies 2–5 and 4 days in study 1. ACEIs were given concurrently with either furosemide or amlodipine in studies 3–5 or added after a 6 and 10-day 'run-in' period in studies 1 and 2, respectively. The percentage suppression from baseline on day 7 (mean ± SD) was 77% ± 15%, and ACE activity was 12.15 ± 3.46 U/L at baseline and 3.29 ± 1.45 U/L on the final day.
*ACE activity was significantly lower (P<0.0001) on the final study day when compared with baseline. ACE: angiotensin-converting-enzyme; ACEI: angiotensin-converting-enzyme inhibitor.

females and 25 intact males. There were 21 Beagles, seven hound crosses and three mixed breed dogs.

There were no significant abnormalities found on physical examination, blood pressure evaluation, complete blood counts, serum chemistries, or urinalyses in any of the studies. Mean ± SD of body weight of the 31 dogs was 15.5 kg ± 10.5 (range 6.7–33.8). The mean age of the dogs was 30.9 months ± 16.1 (range 13.8–53.9). The acclimated dogs from NCSU CVM (studies 1, 2, 3 and 5) were significantly older than the dogs from VA-MD CVM (36.4 months ± 10.1 and 12.1 months ± 1.7, respectively; P<0.001).

Serum ACE activity

Serum ACE activity was measured in 19 dogs from studies 3, 4 and 5. On day 7, approximately 6 hours post-administration, the percentage suppression of serum ACE activity from baseline (studies 3–5; Figure 1) was (mean ± SD) 77% ± 15%. The average serum ACE activity was significantly reduced at the mid-point (days 3/4) and the final time point (day 7) of all studies (P<0.0001). On an individual basis, ACE activity was reduced by more than 50% from baseline in 29/31 (94%) dogs.

Urine aldosterone:creatinine ratio

The baseline UAldo:C values from all dogs in the included studies are shown in Table 2. The mean ± SD UAldo:C from all dogs (n=50) in studies 1–5 was 0.60 ± 0.45 μg/g. On average, the UAldo:C in those dogs undergoing RAAS stimulation with either furosemide or amlodipine increased over the study period, and the mean UAldo:C on the final study day for these dogs was 1.29 ± 0.99 μg/g, significantly greater than baseline values (P<0.0001; Figure 2). In studies 1 and 2, amlodipine and furosemide activation of RAAS preceded ACEI administration. The baseline UAldo:C (mean ± SD) for these 12 dogs was 0.36 ± 0.24 μg/g and the 'activated' baseline UAldo:C (after amlodipine and furosemide administration, yet prior to ACEI initiation) was 0.99 ± 0.78 μg/g and the final day UAldo:C was 0.65 ± 0.31 μg/g (Figure 3(a) and (b)).

There was one instance of incongruous results between centers. When evaluating all dogs enrolled in these studies at baseline, the mean UAldo:C from the 34 NCSU CVM dogs (studies 1, 2, 3 and 5) was 0.40 ± 0.21 μg/g, whereas the mean UAldo:C from the 16 VA-MD CVM dogs (study 4) was 0.99 ± 0.60 μg/g. Eight of the 16 (50%)

Table 2. Determination of the baseline UAldo:C (μg/g) in normal dogs, with the mean + 2 SD as criteria for defining incomplete RAAS blockade.

Studies and institutions included	No. of dogs	Baseline UAldo:C (mean ± SD (μg/g))	UAldo:C (mean +2SD (μg/g))
Studies 1–3 and 5 NCSU CVM	34	0.40 ± 0.21	0.82
Study 4VA-MD CVM	16	0.99 ± 0.56[a]	2.11
All dogs from studies 1–5 NCSU and VA-MD CVMs	50	0.60 ± 0.45	1.50
Esoterix radioimmunoassay	51	0.44 ± 0.26	0.96
Studies1–3, Sayer, 2009,[21] Ames, 2013[25] NCSU CVM			
MSU-DCPAH radioimmunoassay Study 5 NCSU CVM	6	0.48 ± 0.18	0.84

MSU DCPAH: Michigan State University Diagnostic Center for Population and Animal Health; NCSU CVM: North Carolina State University College of Veterinary Medicine; VA-MD CVM: Virginia-Maryland Regional College of Veterinary Medicine; UAldo:C: urine aldosterone:creatinine.
[a]Significantly greater than UAldo:C values obtained from NCSU CVM dogs.

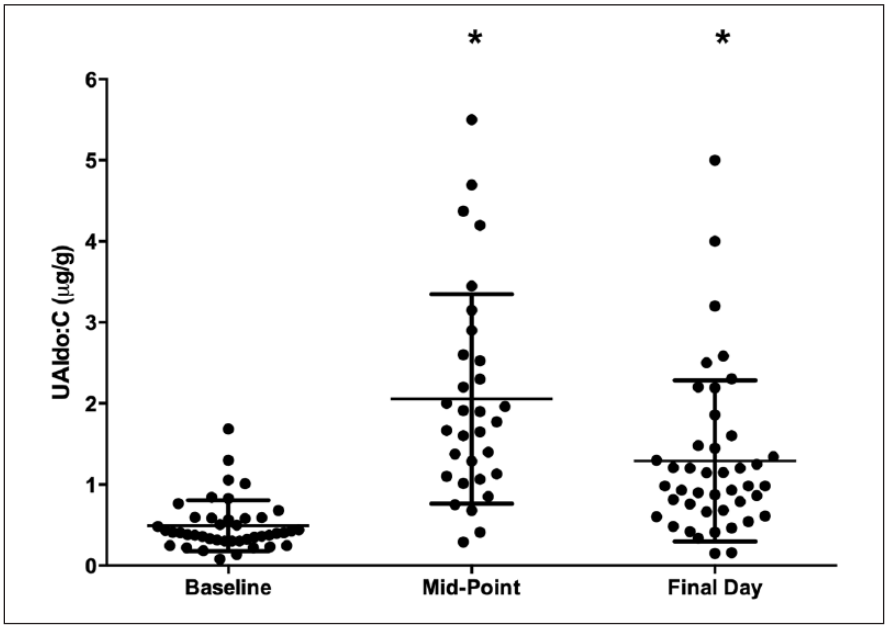

Figure 2. Urine aldosterone to creatinine ratio (UAldo:C in μg/g) in 31 dogs from all five studies.
Scatter dot-plot of the UAldo:C for individual dogs, showing mean ± SD. Baseline and final study day urine samples were collected in all studies. A mid-point sample was obtained 3 or 4 days after initiation of the ACEI in studies 3–5. The duration of ACEI administration on the final day was 7 days in studies 2–5 and 4 days in study 1. ACEIs were given concurrently with either furosemide or amlodipine in studies 3–5 or added after a 6 and 10-day 'run-in' period in studies 1 and 2, respectively. The mean ± SD for the baseline, mid-point and final day time points was 0.49 ± 0.30, 2.06 ± 1.30 and 1.29 ± 0.99 μg/g, respectively.
ACEI: angiotensin-converting-enzyme inhibitor.

dogs from VA-MD CVM had a baseline UAldo:C that exceeded 1.0 μg/g, whereas only one of the 34 (3%) dogs from NCSU CVM had a baseline UAldo:C of 1.0 μg/g and only two dogs had a baseline UAldo:C greater than 0.8 μg/g. The difference in the baseline UAldo:C between the NCSU CVM and VA-MD CMV groups was significant ($P<0.0001$).

When all historical studies from the NCSU CVM were evaluated,[20–24,26,a,e] none of the dogs included in these studies had a UAldo:C greater than 1.0 μg/g at baseline. Values obtained from the two radioimmunoassays were not significantly different ($P=0.30$) and the mean values and cut-off points determined by the mean + 2 SD were similar (Table 2).

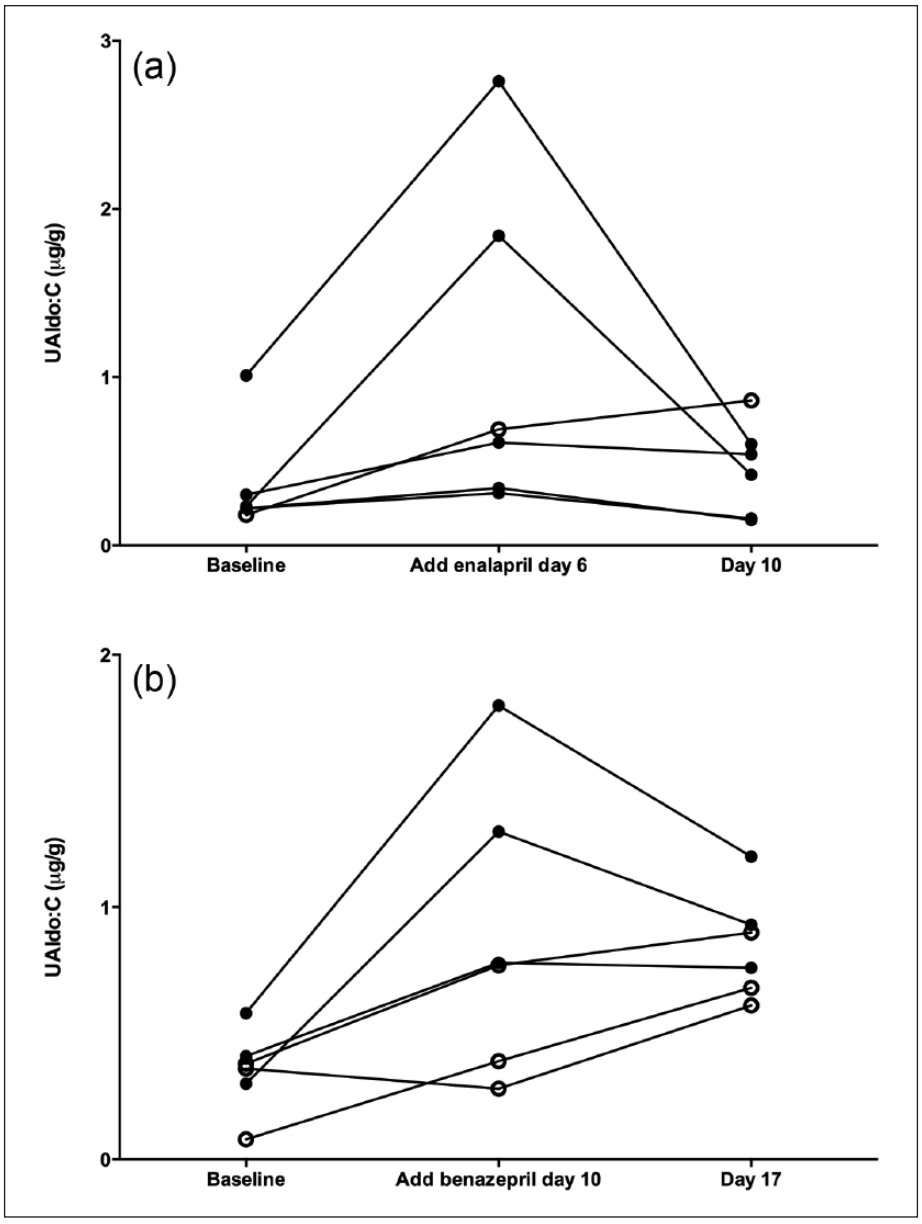

Figure 3. (a) Change in urine aldosterone to creatinine ratio (UAldo:C) for six individual dogs from baseline to the final study day in study 1. Open circles indicate incomplete RAAS blockade (IRB), using definition (a) (see text). The median incidence of IRB was evident in one out of six and none of six dogs, using definitions (a) and (b), respectively. (b) Change in UAldo:C for six individual dogs from baseline to the final study day in study 2. Open circles indicate IRB, using definition (a) (see text). The median incidence of IRB was evident in three of six and one of six dogs, using definitions (a) and (b), respectively.

IRB during ACEI administration

The incidence of IRB in these subacute (7–17 days) studies was determined for the 31 dogs, treated with an ACEI (enalapril or benazepril), using the definitions noted above (Table 1). The incidence of IRB, defined in dogs receiving ACEI after pharmacologic RAAS activation was 33% and 8% for definitions (a) and (b), respectively. The incidence of IRB in dogs receiving ACEI concurrently with pharmacologic RAAS activation was 65% and 61% for definitions (a) and (b), respectively. The overall incidence of IRB was 56%, using the baseline definitions and 47% using the cut-off definition.

The incidence of IRB in the amlodipine-activated RAAS studies was lower than that in the furosemide-activated RAAS for definitions (a) and (b) (P=0.008 and P=0.0021, respectively). The incidence of IRB was similar for both ACEIs (enalapril and benazepril; P>0.05). There was no difference in the incidence of IRB between the high dosage ACEI groups and the standard dosage ACEI groups

(study 1 compared with study 5c; study 3 compared with 5b; study 4 compared with 5a; $P>0.05$).

Discussion

When normal dogs underwent furosemide or amlodipine activation of RAAS and were then administered ACEIs, aldosterone production, as indicated by the UAldo:C rose to greater than the 'activated' baseline value in 33% of dogs. When ACEIs were initiated concurrently with pharmacologic RAAS activation, the UAldo:C rose to a value greater than 100% over their pre-activation baseline in 65% of dogs. This illustrates IRB, which occurred with both stimuli (furosemide and amlodipine) and both ACEIs (enalapril and benazepril) in this subacute model of pharmacologic RAAS activation. Neither high dose enalapril nor high dose benazepril decreased the incidence of IRB in this subacute, experimental model.

For this study of pharmacologic RAAS activation in normal dogs, we evaluated two criteria to define a dog as having experienced IRB, depending upon whether the ACEI was initiated concurrently with, or after pharmacologic RAAS activation. If they had a baseline UAldo:C value after initiation of either furosemide or amlodipine (but before ACEI initiation), this was considered an 'activated' baseline value. If pharmacologic RAAS activation and initiation of ACEI occurred concurrently, their baseline value was their pre-study (days –2 and –1) averaged UAldo:C. For the former group, IRB was defined when a dog had a UAldo:C (a) greater than its own 'activated' baseline or (b) greater than a pre-determined cut-off value of 1.0 μg/g. For the latter group, IRB was defined by a UAldo:C (a) two times greater than its own 'pre-activation' baseline or (b) greater than a predetermined cut-off value of 1.0 μg/g. The cut-off value was derived from the mean + 2 SD of the pre-treatment UAldo:C of dogs from the NCSU CVM[20–23,25, a] whose UAldo:C were determined using the original radioimmunoassay[b] (Table 2). The rationale for selecting this group of dogs was that they were all housed in the same environment and their UAldo:C had been measured by the same laboratory. These definitions were modeled after those used for ABT in people. When compared to those ABT definitions, the definitions used herein were equally or more stringent. To account for within and between-subject coefficients of variation, one recent study defined the related phenomenon of ABT as an increase in blood aldosterone concentration greater than 10% over baseline.[35,36] When this definition is applied to the dogs in studies 1 and 2 with an 'activated' baseline UAldo:C, the incidence of IRB did not change. For dogs in studies 3–5, our 'baseline' definition of IRB was much more stringent, requiring a twofold increase over baseline, as these dogs did not have an activated baseline. In theory, aldosterone excretion should be substantially reduced from baseline with ACEIs and, based on the definitions used in this study, approximately half of dogs in our studies experienced IRB.

The subgroup analyses suggest that there is no difference in the incidence of IRB with either high versus standard dosages of ACEIs or enalapril versus benazepril. Unfortunately, the small number of dogs in these analyses makes it difficult to draw firm conclusions, and larger studies are needed. Questions regarding high dosage and synergy of RAAS blockers have been evaluated in people with proteinuric kidney disease. Although higher dosages of ARBs (and possibly ACEIs) may lead to clinical benefit such as reduced proteinuria,[37] they may not lead to a reduction in the incidence of ABT.[38] It also appears that ACEIs, ARBs and/or direct renin inhibitors may not be synergistic in preventing ABT or reducing proteinuria.[32,39] The lower incidence of IRB in amlodipine-activated as compared to furosemide-activated RAAS studies indicate that a pure vasodilator may be less stimulatory to RAAS than a loop diuretic, with its attendant decrease in blood volume, total body sodium and possibly direct stimulation of RAAS.[40]

The UAldo:C is likely to be a better indicator of IRB/ABT than measurement of a single blood aldosterone concentration, as it combines two (morning and evening) urine samples and gives a better 'average', as each urine sample reflects a longer period (typically hours) of aldosterone secretion. As there is substantial minute-to-minute variation in the circulating levels of RAAS components, resulting from changes in sympathetic nervous system activity and posture, the gold standard for the evaluation of aldosterone excretion is continuous blood sampling.[41–46] This is a time-consuming method and impractical for clinical use. Urine aldosterone secretion is an alternative method of evaluating RAAS activity, with the 24-hour urine aldosterone concentration being the gold standard for measurement.[41] The use of a radioimmunoassay to measure urine aldosterone in the dog has been validated under both resting conditions and physiologic perturbations such as high and low salt intake and furosemide administration.[27] These perturbations resulted in expected changes in both urine aldosterone concentration and UAldo:C measurements, using pooled urine (approximately 1 litre collected over 24 hours) samples. Fortunately, spot urine sampling and the UAldo:C, determined from a single urine sample, has also been validated to correlate with 24-hour urine sampling at both resting conditions and during pharmacologic activation of RAAS.[27] The correlation between 24-hour and spot urine samples after furosemide activation of RAAS was not evaluated, yet further validation comes from subsequent studies of furosemide activated RAAS,[21,22,25] in which the UAldo:C results from a mixed morning and evening urine sample mirror those from the validation study, which used a pooled urine sample.[27] For both human and canine patients, urine sample(s)

can be attained 'free-catch' in the home environment and frozen, reducing patient impact, particularly the stress of a hospital visit for canine patients.

It is important to note that the earliest that ABT has been studied in human patients is after 4 weeks of therapy. Using the antiproteinuric effect of ACEIs/ARBs as a metric for efficacy, these drugs may take several weeks to reach maximal effect. This argues that fair assessment of ACEIs/ARBs should be performed at least several weeks after initiation of therapy. As shown in our model, IRB appears to occur during subacute ACEI administration, but its persistence is not currently known. This remains a compelling question, as aldosterone excretion is never suppressed in some dogs during the course of these subacute studies. In summary, an elevated UAldo:C, despite RAAS blockade in the subacute (<4 weeks) period is best termed IRB, whereas it would be called ABT if used chronically (months). It is also possible that IRB may foretell ABT in human and animal patients.

A definition for IRB/ABT that uses an individual's baseline value has several benefits. First, it is independent of assay and only requires that the same assay be used serially. Second, it allows for customization to the individual rather than using a population-based cut-off value. A cut-off definition, however, is useful when baseline data are lacking, as is often the case in clinical patients. Because of variation in availability/interest in aldosterone testing and in therapies initiated prior to presentation, meaningful baseline samples are not always possible. Therefore, for clinical use, the use of more than one (baseline and cut-off) is likely to be necessary. A patient with acute heart failure probably has significant RAAS activation and while a baseline definition is still possible, the diagnostic criterion (definition) should be less stringent (i.e. ABT diagnosed by UAldo:C after initiation of an ACEI greater than baseline, rather than requiring a two-fold increase over baseline) and with knowledge of the UAldo:C in the normal population. Also, ABT and worsening heart failure, with resultant hypotension and progressively uptitrated dosages of diuretics, can each lead to chronic, maladaptive stimulation of RAAS.[47] While a diagnosis of ABT will not differentiate between these two causes of RAAS activation, patients may benefit from ongoing RAAS monitoring with optimization/tailoring of their RAAS blocking pharmacotherapy.

The dogs from VA-MD CVM had a significantly higher UAldo:C than dogs from NCSU CVM. This finding may reflect differences in dog age (dogs at VA-MD CVM were significantly younger) and differences in conditioning, as the younger dogs had less handling and were observationally more prone to stress/excitement with handling. In people, aldosterone secretion decreases slightly with age, yet remains relatively constant between 1 and 60 years of age.[48] Similar chronobiology in dogs has not been described and it cannot be determined if an age-related difference in aldosterone secretion played a role in the differences between institutions. Subjectively, the dogs with higher UAldo:C were less socialized, more stressed and more excitable. It is important to emphasize that this difference did not alter the results and conclusions of this report. Also, the baseline UAldo:C results from both radioimmunoassays were compared and were not found to be different. Finally, although the type of dog food was uniform between institutions, the quantity of food was not standardized and it is possible that the dogs at VA-MD CVM received relatively less dietary sodium.

Serum ACE activity was measured in studies 3–5 of this report. The magnitude of ACE suppression was highly significant in all studies (percentage suppression from baseline on day 7 (mean ± SD) was 77% ± 15%). Maximal suppression of ACE activity after oral ACEI administration in the dog is rapid and occurs within 1–3 hours.[49–51] For both enalapril and benazepril, this suppression is long-lasting, and there is likely to be little difference in ACE activity between 3 and 6 hours.[49] Less is known for enalapril, however, and it is possible that a 6 hours sample does not represent maximal ACE activity suppression. Despite significant suppression of ACE activity, over half of the dogs in these three studies experienced IRB, regardless of the definition used.

Although the data from these studies suggest a high incidence of IRB with ACEIs in normal dogs receiving pharmacologic RAAS activation, there are multiple well-designed clinical trials demonstrating the benefits of ACEIs on survival and quality of life in dogs with heart failure.[15–18] One possible explanation for the incongruent finding of the efficacy of ACEIs in these canine heart failure patients, is that not all dogs with IRB develop ABT. Also, ABT is reported to occur in 'only' approximately 40–50% of human cardiac patients.[31] In those that experience ABT, there may at least be temporary, or partial suppression of circulatory RAAS and the benefit to those not experiencing ABT may be enough to provide a population benefit. Furthermore, as in the case for some people, there may be up to a year of effective RAAS suppression prior to ABT, accruing patient and, therefore, population benefit during that period of time. Suppression of tissue RAAS may also help to explain the positive results of the above-mentioned studies, as blockade of tissue RAAS compromises tissue-produced angiotensins and aldosterone, thereby giving benefit, even when ABT in the circulation is recognized. Tissue RAAS is, unfortunately, difficult to evaluate in patients, as a tissue homogenate is required to measure its components. ACEIs have variable, and incompletely understood efficacy in their disruption of tissue RAAS due to variations in tissue retention, tissue penetration, half-life and bioavailability.[52,53] The data reported here were generated in the study of the circulating RAAS by means of the UAldo:C and serum ACE activity.

Evaluation of tissue RAAS was not performed, as it would have required myocardial, renal, or vascular tissue homogenates. The mechanisms of IRB/ABT are not known, and may vary between patients with cardiovascular and renal disease. Compelling evidence pertaining to the mechanism comes from patients with diabetic nephropathy and implicates long-term feedback mechanisms after chronic antihypertensive therapy and sodium restriction.[35]

The authors propose that definition (a), using the patient's baseline UAldo:C, be used for IRB/ABT when baseline (prior to initiation of ACEIs/ARBs and ideally, diuretics) aldosterone excretion data are available. If baseline data are not available, a UAldo:C cut-off value can be used and should be determined by evaluation of a normal cohort that is reflective of the patient population. As RAAS activation probably increases incrementally with the progression of heart disease, the attendant decline in cardiac output, and with increasing diuretic dosages, monitoring of an individual's serial UAldo:C may aid in the optimization/titration of RAAS blocking therapies.

We recognize that there are weaknesses in the studies reported here. These studies were carried out in healthy dogs and are subacute in nature. This obviously differs from the clinical situation of chronic heart failure. It can be argued, however, that the incidence of IRB/ABT would increase with the progression of cardiac dysfunction, heart failure and aldosterone-stimulating off-loading therapies. However, the limitation of studies being subacute allows for the possibility that IRB in our model is temporary, with aldosterone excretion diminishing in the first month of ACEI or ARB therapy, thereby invalidating our conclusion that ABT occurs early in the treatment with ACEIs. Another weakness is that we evaluated only circulating RAAS, using urinary aldosterone concentration and serum ACE activity, so questions regarding the RAAS in its entirety cannot be answered. It is possible that the ACEI-induced reduction in angiotensin II levels and resultant preferential dilation of the efferent arteriole and fall in glomerular filtration rate may potentiate intra-renal (tissue) RAAS and lead to increased aldosterone secretion. This might lead to an overestimation of IRB/ABT in this subacute model. Finally, UAldo:C values probably rise with cardiac disease progression and the diagnostic criteria presented here, and those in use in human patients, do not allow for differentiation between the relative roles of failure of RAAS blockade and progression of disease in IRB/ABT.

Despite these weaknesses, we are able to draw some conclusions regarding the frequency of IRB in an experimental model of treated heart failure. Using this model, we have evaluated the effect of various dosages and regimens of ACEI therapy in normal laboratory dogs to determine the incidence of IRB in dogs in which RAAS is activated by diuretics or vasodilators. We conclude that in this model, IRB occurs in a substantial percentage of normal dogs with activated RAAS and receiving ACEIs and that it occurs early in the period of ACE inhibition. These findings suggest that IRB may occur in cardiac, renal and hypertensive veterinary patients, as well, and that this should be studied. Finally, serial evaluation of a patient's UAldo:C may be useful in optimizing pharmacotherapy of heart failure.

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Notes

[a]Lantis AC and Atkins CE. Unpublished data, 2008
[b]Esoterix Laboratories, Calabasas, CA, USA
[c]Coat-a-count aldosterone (RIA), Siemens Medical Diagnostic Solutions, Los Angeles, CA, USA
[d]Michigan State University Center for Population and Animal Health, Lansing, MI, USA
[e]Ames MK and Atkins CE. Unpublished data, 2012
[f]Iams ProActive MiniChunks, Dayton, OH, USA
[g]Norvasc (Amlodipine), Pfizer Inc., New York, NY, USA
[h]Enacard (Enalapril), Merial Ltd., Duluth, GA, USA
[i]Salix (Furosemide), Intervet Canada, Whitby, ON, Canada
[j]Fortekor (Benazepril), Novartis Pharmaceuticals Co., Eschborn, Germany
[k]Vasotec (Enalapril), Valeant Pharmaceuticals LLC, Bridgewater, NJ, USA
[l]Furosemide 12.5 mg and 50 mg, Vedco Inc., St Joseph, MO, USA
[m]Furosemide 20 mg, Mylan Pharmaceuticals Inc., Morgantown, WV, USA
[n]Lotensin (Benazepril), Validus Pharmaceuticals LLC, Parsippany, NJ, USA
[o]ACE REA kit, 01-RK-ACD; ALPCO Diagnostics, Salem, NH, USA
[p]SAS, version 9.4, SAS Institute Inc., Cary, NC, USA

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