Effect of furosemide and high-dosage pimobendan administration on the renin-angiotensin-aldosterone system in dogs

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Objective—To determine whether a high dosage of pimobendan, when administered concurrently with moderate-dosage furosemide to healthy dogs, would activate the renin-angiotensin-aldosterone system (RAAS) more than furosemide alone.

Animals—12 healthy dogs.

Procedures—6 dogs received furosemide (2.0 mg/kg, PO, q 12 h) only, as an RAAS activator, for 10 days. The other 6 dogs received furosemide (2.0 mg/kg, PO, q 12 h) and pimobendan (0.6 mg/kg, PO, q 12 h) for 10 days. The effect of these drugs on the RAAS was determined by measurement of the aldosterone-to-creatinine ratio (A:C) in urine collected in the morning and evening of study days –2, –1, 1, 5, and 10.

Results—Although there was an increase in the urine A:C during the study period in both groups, it was significant only for dogs that received both drugs. The urine A:C only differed significantly between groups on day 1, at which time A:C was greater in the group that received both drugs.

Conclusions and Clinical Relevance—High-dosage pimobendan administration neither substantially suppressed nor potentiated the RAAS when administered with furosemide in healthy dogs. (J Am Vet Med Assoc 2013;74:1084–1090)

The RAAS is activated in states of decreased cardiac output, such as heart failure. Short-term activation of the RAAS, early in the course of heart failure or with volume contraction, is a useful compensatory mechanism, whereas chronic activation of the RAAS becomes maladaptive. The role of the RAAS as a cause of fluid retention, endothelial and baroreceptor dysfunction, and vascular and myocardial remodeling in patients with chronic heart failure is well accepted.1–6 The RAAS can also be activated pharmacologically by drugs often used to treat heart failure. These drugs include vasodilators such as amiodipine7 and hydralazine8 and diuretics such as furosemide.9–11,a,b

Administration of the inodilator pimobendan, when used with conventional heart failure treatments, results in clinical benefits and increased survival times in dogs with heart failure resulting from dilated cardiomyopathy or mitral valve insufficiency.12–14 The positive inotropic effect of pimobendan results from both sensitization of cardiac troponin C to calcium and PDE III inhibition, whereas the vasodilatory effect of pimobendan results primarily from PDE III inhibition.15–17 Pimobendan induces both venodilation and arteriolar dilation in dogs with propranolol-induced myocardial depression.18 Because of its vasodilatory effect, pimobendan has the potential to activate the RAAS.

The effect of pimobendan on the furosemide-induced RAAS in healthy dogs has been evaluated in the authors’ laboratory.11,19 In those studies, as with the present study, furosemide (as an RAAS stimulant) was administered at a dosage of 2 mg/kg, PO, every 12 hours. At a high dosage (0.5 mg/kg, PO, q 12 h), pimobendan potentiated furosemide-induced activation of the RAAS in the short-term (3 days),19 but this did not occur when the recommended dosage (0.25 mg/kg, PO, q 12 h) was administered for 10 days.11 A logical step
forward from those studies was the evaluation of the effect of high-dosage pimobendan (0.6 mg/kg, PO, q 12 h) on the furosemide-activated RAAS of healthy dogs over a longer time period (10 days), to confirm or refute our earlier findings. This dosage, chosen to be high but clinically relevant, is twice the highest dosage recommended by the American College of Veterinary Internal Medicine consensus panel for the treatment of chronic valvular disease in dogs (0.25 to 0.3 mg/kg, PO, q 12 h [some panelists suggested a dosage of 0.25 to 0.3 mg/kg, PO, q 8 h in the case of refractory heart failure]).

Clinical relevance of the dosage used in the present study was established by evaluation of 116 dogs with refractory heart failure that received pimobendan as outpatients at least 3 times daily at the authors’ institution from 2007 to 2011. It was determined that 65.5% of these dogs received > 0.9 mg/kg/d, with a mean dosage of 1.23 mg/kg/d (range, 0.9 to 2.69 mg/kg/d), more than twice the manufacturer’s and American College of Veterinary Internal Medicine consensus panels’ recommendation. The use of such dosages illustrates the clinical importance of determining whether high-dosage pimobendan potentiates the effect of furosemide on the RAAS.

Therefore, the purpose of the study reported here was to determine whether administration of a high dosage of pimobendan, an inodilator, further stimulates the RAAS in dogs in which the RAAS had been activated by furosemide, by measurement of the urine A:C, a surrogate for 24-hour urine aldosterone excretion.

**Materials and Methods**

**Animals**—Twelve mature purpose-bred dogs > 1 year of age were enrolled in the study. Each dog was determined to be healthy by evaluation of history, a complete physical examination, and analysis of a data-base consisting of systemic BP, CBC, serum biochemical profile, and urinalysis. The dogs were housed in a facility approved by the Association for Assessment and Accreditation of Laboratory Animal Care International. The light-dark cycles were controlled, and dogs received a standard commercial diet (0.42% sodium and 0.84% chloride on a dry-matter basis). The North Carolina State University College of Veterinary Medicine Institutional Animal Care and Use Committee approved this study.

**Study design**—Dogs were allocated into 2 groups of 6. The allocation was random with regard to individual dogs, but selection was performed to provide equal numbers of males and females and equal numbers of Beagles and crossbred dogs in each group. Group F received furosemide (2 mg/kg, PO, q 12 h) for 10 days. Group F + P received furosemide (2 mg/kg, PO, q 12 h) and pimobendan (0.6 mg/kg, PO, q 12 h) for 10 days. Each dog served as its own control for intra-group comparisons and was paired with another dog for intergroup comparisons. All dogs were tested contemporaneously to minimize the chances that uncontrolled-for environmental changes over time influenced the results. A placebo group was not used because the main focus of the study was to compare the effects of furosemide alone with those of furosemide plus pimobendan, whereas change over time from baseline was of secondary interest. Drug administration began on day 0. To measure the time course and magnitude of the RAAS response to furosemide and pimobendan, systemic BP was measured and twice-daily (morning and evening) samples for urine A:C were obtained on days –2, –1, 1, 5, and 10 of the study. Body weight and HR were also measured on these 5 days. In addition, a serum biochemical panel (phosphorus, magnesium, sodium, potassium, calcium, chloride, albumin, urea nitrogen, creatinine, and bicarbonate) and CBC (with Hct and plasma protein concentration) were performed on days –2, 5, and 10.

**Measurement of BP and A:C**—After allowing the dogs to acclimate, oscillometric BPs was obtained with cuff placed over the coccygeal artery. The mean of 3 consecutive measurements that varied < 10% was calculated to determine systolic, mean, and diastolic BP.

Five milliliters of urine was obtained by either free catch or cystocentesis from each dog as described. Each sample was refrigerated immediately and frozen at –70°C within 3 hours of collection. One day following study completion, equal aliquots of each subject’s morning and evening urine samples were thawed, mixed, refrozen, and submitted for determination of urine aldosterone concentration (both free aldosterone and the metabolite, aldosterone glucuronide) via radioimmunoassay and urine creatinine concentration, which allowed calculation of the urine A:C.

**Statistical analysis**—Normal probability plots revealed that BP, BW, HR, urine creatinine concentration, urine aldosterone concentration, calculated urine A:C, and results of all serum biochemical and CBC analyses followed a normal distribution. Accordingly, these were analyzed by use of a mixed-model ANOVA to assess the effect of time point within treatment and the effect of treatment grouping at baseline and at each time point during administration of medications. The linear model included treatment, time, and treatment × time as fixed effects, with dog identification as the random effect. For each model, residual plots were inspected to verify model adequacy (ie, that the errors followed a normal distribution with constant variance). A paired t test was used to compare prestudy variables (eg, HR and BW) between groups. Baseline values for BW, HR, and urine A:C were calculated as the mean of samples obtained on days –1 and –2. Baseline values for serum biochemical and CBC variables were obtained on day –2. For all comparisons, values of P < 0.05 were considered significant. All analyses were performed with statistical software.

**Results**

**Dogs**—Group F consisted of 2 sexually intact females and 4 sexually intact males, and group F + P consisted of 1 sexually intact female, 1 spayed female, and 4 sexually intact males. There were 8 Beagles, 3 Labrador Retriever crosses, and 1 mixed-breed dog. No clinically relevant abnormalities were found in any dogs upon physical examination, BP evaluation, CBC,
significant decreases in serum potassium (group F vs. F + P, \( P = 0.003 \)) on day 10 compared with that of group F (Table 1).

Serum biochemical analysis, or urinalysis. Mean ± SD BW of dogs in group F was \( 15.6 ± 9.8 \) kg. Mean ± SD BW of dogs in group F + P was \( 15.5 ± 8.4 \) kg. Mean ± SD age of dogs in group F was 50.8 ± 20.1 months and in group F + P was 38.2 ± 17.4 months (\( P = 0.271 \)). Mean ± SD dosage of furosemide for dogs in group F was 2.12 ± 0.07 mg/kg, PO, every 12 hours. Mean dosages for furosemide and pimobendan for dogs in group F + P were 2.17 ± 0.12 mg/kg and 0.625 ± 0.015 mg/kg, PO, every 12 hours, respectively.

BW, BP, and HR—No significant change in BW, systolic BP, or diastolic BP within or between groups was detected during the study (Table 1). On days 1 and 5, systolic BP in group F + P was less than that in group F but not significantly so (\( P = 0.09 \) and \( P = 0.06 \), respectively). Heart rate increased significantly (\( P = 0.01 \)) in group F + P during the study period but did not differ significantly between the 2 groups.

Serum biochemical analyses and CBCs—No significant differences in concentrations of BUN, phosphorus, sodium, or anion gap were detected during the study period. A significant (\( P = 0.02 \)) increase in serum creatinine concentration over time was detected in group F, and serum creatinine concentration was significantly (\( P = 0.03 \)) greater in group F than in group F + P on day 5. Significant (\( P < 0.001 \)) but inconsistent changes in serum calcium and albumin concentrations were detected in both groups, although no values differed significantly between groups. Both variables were decreased relative to baseline values on day 5 but were increased relative to baseline values on day 10. Serum bicarbonate concentration increased over time in group F (\( P = 0.003 \)) and group F + P (\( P = 0.03 \)) and was significantly (\( P = 0.01 \)) greater in group F + P on day 10 compared with that of group F + P (Table 1).

Fig. 1—Mean ± SD values for urine A:C in healthy dogs (6/group) administered furosemide (white bars) or furosemide and pimobendan (black bars) for 10 days. *Significant (\( P < 0.05 \)) difference between groups at that time point. BL = Baseline.

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Table 1—Mean ± SD values for variables in clinically normal dogs (6/group) administered furosemide (group F) or furosemide and pimobendan (group F + P) for 10 days.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>Day 1</th>
<th>Day 5</th>
<th>Day 10</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (kg)</td>
<td>15.5 ± 9.6</td>
<td>14.9 ± 9.0</td>
<td>15.3 ± 9.6</td>
<td>15.2 ± 9.3</td>
<td>N/A</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>119 ± 17</td>
<td>127 ± 20</td>
<td>122 ± 16</td>
<td>118 ± 21</td>
<td>125 ± 16</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>142.3 ± 9.7</td>
<td>147.3 ± 18.4</td>
<td>153.3 ± 5.9</td>
<td>143.2 ± 15.8</td>
<td>137.2 ± 21.6</td>
</tr>
<tr>
<td>Sodium (mEq/L)</td>
<td>144.7 ± 2.0</td>
<td>N/A</td>
<td>145.5 ± 1.1</td>
<td>143.2 ± 1.2</td>
<td>144.5 ± 0.5</td>
</tr>
<tr>
<td>Potassium (mEq/L)</td>
<td>4.73 ± 0.34</td>
<td>N/A</td>
<td>4.22 ± 0.31*</td>
<td>4.18 ± 0.21*</td>
<td>3.95 ± 0.24*</td>
</tr>
<tr>
<td>Chloride (mEq/L)</td>
<td>111.3 ± 2.4</td>
<td>N/A</td>
<td>107.2 ± 1.7*</td>
<td>106.8 ± 1.2*</td>
<td>108.7 ± 1.8*</td>
</tr>
<tr>
<td>Bicarbonate (mEq/L)</td>
<td>23.1 ± 3.6</td>
<td>N/A</td>
<td>26.2 ± 2.1*</td>
<td>27.3 ± 2.3*</td>
<td>26.2 ± 2.1*</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>10.84 ± 0.16</td>
<td>N/A</td>
<td>9.42 ± 0.34*</td>
<td>10.48 ± 0.32*</td>
<td>9.53 ± 0.32*</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.94 ± 0.15</td>
<td>N/A</td>
<td>0.95 ± 0.08*</td>
<td>0.88 ± 0.12</td>
<td>0.87 ± 0.18*</td>
</tr>
<tr>
<td>Albumin (mg/dL)</td>
<td>3.53 ± 0.15</td>
<td>N/A</td>
<td>3.37 ± 0.10*</td>
<td>3.70 ± 0.27</td>
<td>3.23 ± 0.28*</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>49.8 ± 5.8</td>
<td>N/A</td>
<td>54.7 ± 4.5t</td>
<td>54.2 ± 6.8t</td>
<td>50.3 ± 3.8</td>
</tr>
<tr>
<td>TS (g/dL)</td>
<td>6.6 ± 0.3</td>
<td>N/A</td>
<td>7.1 ± 0.4</td>
<td>7.2 ± 0.3</td>
<td>7.1 ± 0.4</td>
</tr>
</tbody>
</table>

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*Values are significantly (\( P < 0.05 \)) different from baseline value for that group. †Values are significantly (\( P < 0.05 \)) different between groups at that time point. N/A = Not available.
Discussion

Because the pharmacotherapy of heart failure usually includes furosemide and often amloidipine, both known to activate the RAAS, it is important to determine whether commonly coadministered drugs such as the inodilator pimobendan potentiate this activity. In the present study, dogs treated with furosemide (approximately 4 mg/kg/d), with and without high-dosage pimobendan (approx 1.2 mg/kg/d), were evaluated for evidence of activation of the RAAS via measurement of the urine A:C. In both groups, the A:C increased approximately 2-fold over time; in group F, the increase neared significance (P = 0.09) and the difference from baseline approached significance on day 5 (P = 0.056). However, a significant difference between groups was only detected on day 1. The increase in A:C over time in the F + P group was significant. We interpret these findings as evidence of stimulation of the RAAS by furosemide, as in previous results from our laboratory. The A:C was significantly higher in the F + P group, compared with the F group, only on day 1, suggesting that potentiation of furosemide’s RAAS activation by pimobendan was brief.

Results of previous studies in the authors’ laboratory and those of the present study suggest that, at the recommended dosage, pimobendan does not potentiate furosemide-induced RAAS activation, and at high dosages, it causes a mild, early, and transient increase in RAAS activation in clinically normal dogs receiving furosemide.

Activation of the RAAS can be evaluated via measurement of urinary aldosterone excretion, ideally during a 24-hour period. An assay to measure urine aldosterone in dogs has been validated, and the urine A:C, measured by use of spot tests (morning and evening urine samples) and radioimmunoassay methodology, has been correlated with 24-hour urine aldosterone excretion. This assay measures both free aldosterone and one of its more abundant metabolites, aldosterone glucuronide. Further validation of the urine A:C as a measure of RAAS activation is in the expected response of the urine A:C to perturbations, such as volume reduction, BP reduction, changes in sodium intake, and stress. In addition, the higher the urine A:C in canine heart failure patients, the poorer the prognosis. The consistency and reliability of this method is likely enhanced by increased frequency of sampling, with each urine sample having the potential to reflect several hours of aldosterone secretion. This method is superior to spot test blood measurements of RAAS components because of substantial minute-to-minute variation that occurs in plasma with changes in sympathetic stimulation and posture changes. The minute-to-minute variation in blood samples can be eliminated by continuous 24-hour collection of blood (the gold standard for evaluation of aldosterone excretion) with an indwelling catheter, but this is cumbersome and therefore not clinically useful.

Results of the present study suggested that the effect of high-dosage pimobendan on the RAAS in dogs receiving furosemide was of low magnitude and short duration. There may be several possible explanations for these results. First, it is possible that the magnitude of vasodilation induced by pimobendan was not sufficient to cause substantial stimulation of the RAAS. Although the dogs receiving pimobendan and furosemide had, typically, lower systolic BP on days 1 and 5, compared with dogs receiving furosemide alone, this difference was not significant and no dog had a clinically important decrease in BP. Second, the positive inotropic effect of pimobendan may offset any decrease in renal blood flow or GFR caused by vasodilation and reduced glomerular pressure, thereby decreasing the stimulation for renin release from the kidney (ie, RAAS activation). This inotropic effect does not, however, lead to suppression of the furosemide-induced RAAS in healthy dogs. Another possible explanation is that the positive inotropic effect of pimobendan may lag behind the vasodilatory effect, allowing the PDE III–induced vasodilatory effect to predominate early (during the first few days after administration), with reversal when either pimobendan’s inhibition of PDE III and resultant vasodilation plateaus or the drug’s positive inotropic effect catches up, to offset the vasodilatory effect on renal blood flow with its resultant renin release.

Though of only modest proportions, the increase in serum creatinine in group F but not group F + P on day 5 suggests that furosemide-induced prerenal azotemia may have been partially offset by improved cardiac output induced by pimobendan’s positive inotropic effect. Previous studies in the authors’ laboratory revealed significantly lower BUN in clinically normal dogs treated with pimobendan and furosemide, compared with that in dogs receiving furosemide alone. A study of pimobendan administration in dogs with experimentally induced mitral regurgitation revealed a significant increase in renal blood flow as well as a slight increase in GFR at 2 and 4 weeks of standard-dose pimobendan administration. Conversely, a study of healthy dogs found no significant difference in GFR between those receiving standard-dose pimobendan (0.25 mg/kg, PO, q 12 h) and a control group. The findings suggesting improved renal function with pimobendan administration are supported by a number of hemodynamic studies. A study of the hemodynamic effects of pimobendan in humans with heart failure found that this inodilator improved cardiac output, thereby increasing renal blood flow. Pimobendan administration improves cardiac output in healthy, anesthetized dogs and in conscious dogs with propranolol-induced myocardial depression and improves indices of the left ventricular inotropic and lusitropic function in conscious dogs with pacing-induced heart failure.

Mean BW decreased in both groups during the study period, with the greatest change observed on day 1 (Table 1). This change was not significant within the groups, and there was no difference between groups. Although water intake and urine output were not directly quantified in this study, the lack of a significant change in mean BW and a subjectively increased urine output in all dogs suggested that water intake was increased. The lack of a significant progressive decrease in BW during the study period was also compatible with reduced diuresis over time, associated with the braking phenomenon, in which the magnitude of natriuresis following repeated diuretic doses decreases...
over time as solute and fluid reabsorption in the proximal tubules increases. Renin-angiotensin-aldosterone system activation and sympathetic nervous system activation are also thought to play a role in the braking phenomenon and diuretic resistance. Conversely, in short-term diuretic administration and volume contraction, mechanisms such as increased proximal tubule solute and fluid retention, postdiuretic sodium retention, and increased thirst may also mitigate RAAS activation and contribute to the plateau seen in furosemide-induced RAAS activity in healthy dogs. Long-term mechanisms of diuretic resistance such as changes in structure and function of the distal portion of the nephron likely did not occur in this short-term study in clinically normal dogs.

The decrease in serum potassium and chloride concentrations and increase in Hct, plasma protein, and albumin concentration detected in this study were consistent with the administration of a loop diuretic (Table 1). It is unlikely that these changes were caused by high-dose pimobendan administration because there was no difference in these variables between the 2 groups. Relative to baseline, the serum calcium concentration in both groups decreased between days 1 and 5, then increased to values that were significantly greater than baseline on day 10. The initial decrease in serum calcium concentration was likely caused by an increase in urinary calcium loss associated with furosemide administration. The subsequent increase in serum calcium concentration between days 5 and 10 in both groups is more difficult to explain but may have been caused by hemococoncentration. The suggestion of hemococoncentration was supported by a significant increase in Hct and plasma protein concentration and a decrease (albeit nonsignificant) in mean BW in both groups, although BW changes were most pronounced on day 1, not days 5 to 10, when serum calcium concentrations were highest. The initial decrease in serum albumin concentration in both groups is also difficult to explain but this finding was mild and was not thought to be clinically important. The serum bicarbonate concentration increased in both groups over the study period, although the increase was significant only in the F group, and serum bicarbonate concentration was significantly greater in group F only on day 10. This increase in serum bicarbonate concentration and the concurrent hypochloremia were likely caused by diuretic-induced, mild, hypochloremic alkalosis. In patients with heart failure, this is thought to occur primarily through increased renal acid excretion resulting from RAAS activation and increased circulating aldosterone. Volume contraction can also result in an increase in proximal tubular bicarbonate reabsorption as well as enhanced excretion of sodium and chloride without proportional loss of bicarbonate. The development of metabolic alkalosis during furosemide-induced activation of the RAAS has been detected in clinical cases and in a previous study.

The increase in HR seen in group F + P was compatible with a positive chronotropic effect of pimobendan. This may also have been caused by baroreceptor-mediated reflex in response to diuretic administration and resultant volume contraction, possibly potentiated by pimobendan. Pimobendan has a positive chronotropic effect when administered to anesthetized, healthy dogs. The exact mechanism for this increase in HR is not known.

Studies in humans and other animals have found sex differences in the activity of the RAAS in physiologic and pathophysiologic states. Generally, plasma renin concentration and ACE activity are higher in males. Also, estrogen is thought to decrease angiotensin II-stimulated aldosterone secretion. A study in ovariectomized dogs revealed that dogs receiving estrogen had lower plasma aldosterone concentration than dogs not receiving estrogen. The present study included 3 sexually intact females, 1 spayed female, and 8 sexually intact males. Interestingly, the highest urine A:C value in each group on 9 of 10 study days was observed in samples from a female dog (including the spayed female). Unfortunately, given the small size of this study, no conclusions can be drawn regarding a difference in urine A:C between males and females and further study is needed to determine sex differences in this variable. The specificity of the urine aldosterone assay used in this study has been validated, and it is unlikely that cross-reactivity with other steroid hormones substantially affected urine aldosterone measurements.

Limitations of this study included its short duration, which did not mimic the typical long-term administration of drugs used for heart failure in clinical patients. This study also used healthy dogs and pharmacologic induction of the RAAS. Evaluation of the effect of high-dosage pimobendan on the RAAS of patients with naturally occurring heart failure and pathologic and pharmacologic activation of the RAAS is warranted. The healthy dogs used in this study appeared to tolerate furosemide-induced diuresis (which was not measured) and volume contraction well because they did not have a significant decrease in BW or increase in serum creatinine concentration between baseline and day 10 of the study period. This efficient physiologic adaptation to the administration of a loop diuretic may not be reflective of what occurs in dogs with heart disease and abnormal cardiac function. The small number of dogs and intermittent rather than daily sampling of blood and urine were also weaknesses, although the study was mathematically adequately powered. A larger number of dogs and daily sampling would have allowed for a more accurate and continuous depiction of the effect of high-dosage pimobendan on the RAAS. Also, the addition of both positive (pimobendan only) and negative (placebo) control groups would have strengthened this study, allowing evaluation of the potential effects of pimobendan monotherapy on RAAS activation and better detection of the effects of environmental fluxes and alterations in the daily routine of the experimental dogs, including increased human interaction, venupuncture, and cystocentesis. Lastly, although the terminal portion of the RAAS cascade is most important and its activation worsens prognosis in heart failure, we did not evaluate other portions of this cascade (plasma renin, angiotensin I, angiotensin II, and aldosterone concentrations).

In the present study of healthy dogs, high-dosage pimobendan administration (0.6 mg/kg, PO, q 12 h) neither substantially suppressed nor potentiated
furosemide-induced RAAS activation. Pimobendan may help ameliorate furosemide-induced prerenal azotemia.

References

