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Acute Effect of Pimobendan and Furosemide on the Circulating Renin-Angiotensin-Aldosterone System in Healthy Dogs

M.B. Sayer, C.E. Atkins, Y. Fujii, A.K. Adams, T.C. DeFrancesco, and B.W. Keene

Background: The renin-angiotensin-aldosterone system (RAAS) is activated in states of decreased cardiac output and by certain cardiovascular therapeutic agents, such as loop diuretics and vasodilators.

Hypothesis: Short-term treatment with the inodilator, pimobendan, will not activate the circulating RAAS because its vasodilatory action will be offset by its positive inotropic property, thereby ameliorating RAAS stimulation at the juxtaglomerular apparatus. Furthermore, pimobendan will suppress RAAS activation produced by furosemide.

Animals: Nine healthy laboratory dogs were used in this study.

Methods: Experimental, cross-over study. Dogs were administered pimobendan (0.5 mg/kg q12h) for 4 days followed by furosemide (2 mg/kg q12h) and then, after a wash-out period, a combination of the drugs. Aldosterone : creatinine (A : Cr) was measured at the end of each treatment cycle.

Results: There was no significant increase in the average urinary A : Cr with the administration of pimobendan (control urinary A : Cr = 0.46, standard deviation (SD) 0.33; pimobendan A : Cr = 0.48, SD 0.28). There was a significant increase in the average urinary A : Cr after administration of furosemide (urinary A : Cr = 1.3, SD 0.70) and with the combination of furosemide and pimobendan (urinary A : Cr = 2.9, SD 1.6).

Conclusions and Clinical Relevance: Short-term administration of high-dose pimobendan, does not activate the RAAS in healthy dogs. Pimobendan did not prevent RAAS activation associated with furosemide therapy. These results in healthy dogs suggest that furosemide therapy, with or without pimobendan, should be accompanied by RAAS suppressive therapy.

Key words: Furosemide; Heart failure; Inodilator; Pimobendan; Renin-angiotensin-aldosterone system.

The renin-angiotensin-aldosterone system (RAAS) is an important component of the neurohumoral response to decreased cardiac output. RAAS activation has also been documented with the administration of loop diuretics[1,2] and vasodilating drugs such as amlodipine[3] and hydralazine.[4] Although appropriate and useful in states of dehydration, hemorrhage, and shock, in the presence of congestive heart failure and hypertension, chronic RAAS activation contributes to adverse cardiac remodeling and other maladaptive processes.[5–7] Blunting of the RAAS is an integral part of the management of heart disease, hypertension, and renal disease and, given the extensive and potentially adverse effects of the RAAS, it has become increasing important to assess the RAAS response in disease states and with therapeutic strategies.[5,6]

Pimobendan, a benzimidazole-pyridazinone, is both a calcium sensitizer and phosphodiesterase III inhibitor that causes peripheral vasodilation that reduces cardiac afterload and improves cardiac output.[7,8] Pimobendan provides clinical improvement and increased survival times in heart failure attributable to dilated cardiomyopathy and mitral valve insufficiency in dogs.[8–12]

From the College of Veterinary Medicine, North Carolina State University, Raleigh, NC. Dr Sayer is presently affiliated with Carolina Veterinary Specialists, Charlotte, NC. Dr Fujii is presently affiliated with Azabu University, Tokyo, Japan. *Study results were presented at the American College of Veterinary Internal Medicine Forum, June 9, 2007.*

Corresponding author: Dr Margaret B. Sayer, c/o Dr Clarke Atkins, 4700 Hillsborough Street, Raleigh, NC 27606; e-mail: pegbooth@yahoo.com.

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Abbreviations:

A : Cr	aldosterone: creatinine ratio
BP	blood pressure
q12h	every 12 hours
RAAS	renin angiotensin aldosterone system
SD	standard deviation
SUN	serum urea nitrogen
UA	urinalysis

Circulating RAAS activation can be measured by quantification of serum renin, angiotensin II, and aldosterone concentrations or by evaluating urinary aldosterone secretion either by 24-hour urinary aldosterone excretion or by the urinary aldosterone : creatinine ratio (A : Cr). Measuring serum renin, angiotensin, and aldosterone concentrations to evaluate RAAS activation can be problematic because of misleading minute-to-minute variations in serum hormone levels.[13] Urinary aldosterone concentrations provide an ''average'' estimate of aldosterone secretion over several hours as the aldosterone-containing urine pools in the bladder. The urinary A : Cr has been validated and used as an indicator of 24-hour aldosterone secretion.[3,13,14]

We hypothesized that pimobendan would not activate the RAAS because its positive inotropic effect would maintain renal blood flow, offsetting the vasodilatory tendency to activate the RAAS in healthy animals. Furthermore, we tested the hypothesis that pimobendan might blunt furosemide-induced RAAS activation.

Materials and Methods

The study protocol (Fig 1) was approved by the North Carolina State University Animal Care and Use Committee. On day 0, 9 research dogs (7 females and 2 males, average body weight 19.9

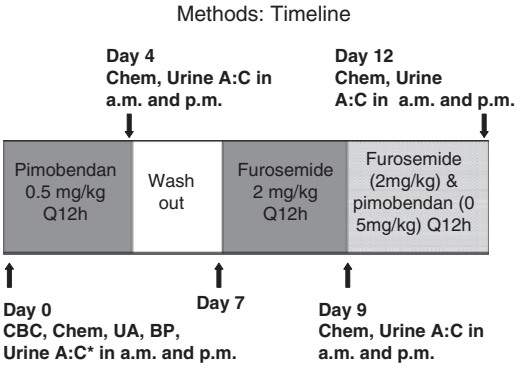

Fig 1. Time line showing sequence of medications and the tests performed at each point in time. Day 0—physical examination and baseline diagnostics (serum chemistry and electrolytes, complete blood count, and urine A : Cr [AM and PM samples mixed]) were gathered. Day 1—Pimobendan administration was initiated and continued for 4 days. Day 4—Pimobendan was administered in the morning, blood was drawn, and morning and evening urine samples were collected, frozen and later mixed for A : Cr determination. The dogs were then allowed a 72-hour wash-out period. On day 7, furosemide administration was begun and continued for 2 days. Blood was then drawn for serum chemistry and electrolyte determination and complete blood count. Morning and evening urine samples were collected, mixed, and frozen, as described above. Pimobendan administration was reinstituted and the combination of furosemide and pimobdendan was continued for 3 days, at which time blood was drawn and urine samples were again collected and handled as described. Chemistry, serum chemistry panel; A : Cr, aldosterone : creatinine; UA, urinalysis; BP, blood pressure determination.

[standard deviation (SD) 7.37] kg), deemed healthy after physical examination, complete blood count, serum chemistry analysis, urinalysis, and blood pressure measurement, were used in this study. Blood pressure measurement was performed oscillometrically with the forelimb.[a] The average of 3 consecutive systolic blood pressure measurements, within 10% of each other and for which the oscillometrically determined heart rate matched the dog's manually determined heart rate, were utilized. Lastly, morning and evening "spot" urine samples were collected for later mixing of equal aliquots and frozen at −70°C, for urinary A : Cr determination, as described previously.[3,13] On day 1 study, the dogs began receiving 0.5 mg/kg of pimobendan[b] (0.50 [SD 0.05] mg/kg q12h). They received 7 doses over 60 hours. On the 4th day, only the morning dose of pimobendan was given and a blood sample was obtained for serum chemistry analysis. Additionally on day 4, morning and evening urine samples were collected and frozen; later, equal aliquots were mixed, refrozen, and submitted for determination of A : Cr. To ensure that the drug was having maximal effect, urine A : Cr was determined from samples taken after at least 5 doses were administered.[c]

After the final administration of pimobendan there was a 72-hour (> 5 half-lives) wash-out period. The dogs then received 2 mg/kg of furosemide[d] (2.0 [SD 0.17] mg/kg) PO twice daily. The dogs received 5 doses of furosemide over 36 hours. On the 4th day, the morning furosemide dose was given and a blood sample was collected for serum chemistry analysis.[e] Morning and evening urine samples were again collected and equal aliquots frozen and later combined for analysis. The dogs continued to receive furosemide at the 2 mg/kg dosage and pimobendan was reinitiated at 0.5 mg/kg q12h. The dogs received 6 doses of this combination over a 60-hour period. At the end of this administration period, blood was collected for serum chemistry evaluation and morning and evening urine samples were collected, as described above.

Each dog acted as its own control. Results are reported as mean and SD. Because of the small number of subjects, a Gaussian distribution was not assumed. Comparisons of values obtained from each treatment phase were carried out by the more conservative Wilcoxon's signed rank test with significance assumed at $P = .05$.

Results

There were no significant abnormalities found on physical examination, complete blood counts, serum chemistry analyses, urinalyses, or blood pressure evaluations. After administration of pimobendan, there was a significant ($P < .01$) but mild, clinically irrelevant, increase in the mean (SD) serum sodium concentration whereas the combination of furosemide and pimobendan produced a likewise clinically unimportant fall in serum sodium concentration ($P < .01$; Table 1). Furosemide administration was associated with a decrease in serum potassium concentration ($P = < .01$; Table 1). The combination of furosemide and pimobendan was also associated with a reduction in serum potassium concen-

Table 1. Values for SUN and electrolytes are displayed for all treatment situations dogs receiving pimobendan (0.5 mg/kg q12h), furosemide (2 mg/kg q12h) and the combination of pimobendan and furosemide.

Treatment	SUN (mg/dL)	Creatinine (mg/dL)	Phosphorous (mg/dL)	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)
Control	16.4	0.84	3.8	147.2	4.1	109.9
	(3.13)	(0.21)	(0.73)	(1.39)	(0.28)	(1.45)
Pimobendan	13.6*	0.82	3.6	149.2*	4.0	110.3
	(2.19)	(0.21)	(0.39)	(1.56)	(0.33)	(1.56)
Furosemide	19.1	0.98*	3.9	147.3	3.6*	104.9*
	(2.98)	(0.29)	(0.21)	(1.80)	(0.48)	(1.80)
Pimobendan + furosemide	20.9*	0.98*	4.0	144.9*	3.7*	102.7*
	(3.62)	(0.27)	(0.52)	(2.15)	(0.39)	(3.31)
Normal values	8–27	0.5–1.6	2–6.7	147–154	3.9–5.2	104–117

Control values are those obtained before drug administration. See text for detailed description.
*$P < .05$, as compared with control values.
SUN, serum urea nitrogen.

tration ($P < .02$). Serum chloride concentration fell ($P < .01$; Table 1), with furosemide alone and with the combination of furosemide and pimobendan.

Pimobendan mildly, but significantly ($P < .05$), lowered the average serum urea nitrogen (SUN), whereas the administration of furosemide with pimobendan increased both SUN ($P < .01$) and serum creatinine concentration ($P < .05$; Table 1). With furosemide alone, a similar increase in serum creatinine concentration was observed ($P < .05$; Table 1). None of these changes were considered to be clinically relevant.

The urinary A : Cr after the administration of pimobendan alone was not significantly different than the control value (Fig 2). Furosemide administration was associated with a significant ($P < .01$) increase in urinary A : Cr, and the combination of furosemide and pimobendan was also associated with a significantly increased urinary A : Cr ($P < .01$ and .004, Fig 3).

Discussion

The results of this study suggest that pimobendan, unlike other vasodilators, does not activate the RAAS in healthy dogs. This is likely because of pimobendan's positive inotropic effect maintaining the glomerular filtration rate, thereby mitigating the drug's potential vasodilatory activation of the RAAS. Furosemide activated the RAAS (Fig 3). Although concurrent administration of pimobendan did not diminish this RAAS activation (Fig 3) and in fact, RAAS activation continued to rise significantly over study days 9–12, during the time in which pimobendan was administered concurrently. It is possible that the combination of furosemide and pimobendan increased RAAS activation by lowering renal blood pressure caused by the respective additive effects of hypovolemia and vasodilation associated with furosemide and pimobendan administration. Another potential interpretation of this finding is a synergistic effect of furosemide and high-dose pimobendan on RAAS activation in the subacute setting by an unknown mechanism. However, the further increase in urinary A : Cr on day 12 (3rd day of pimobendan and furosemide combination therapy) seems more likely because of the additional 3 days furosemide administration. This explanation is supported by the fact that the highest average SUN and serum creatinine concentrations were observed with this combination therapy.

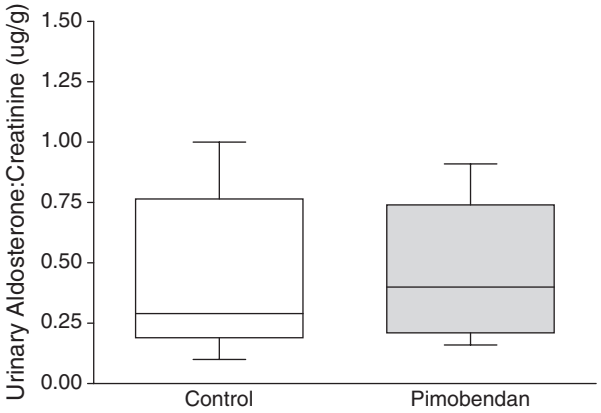

Fig 2. The urinary aldosterone to creatinine ratio (μg/g) before and after the administration of pimobendan (0.5 mg/kg BID) for 4 days. The box and whiskers plot demonstrates the median (line), 25 and 75% confidence limits (box), and range (bars). There is no evidence of activation of the circulating renin-angiotensin-aldosterone system.

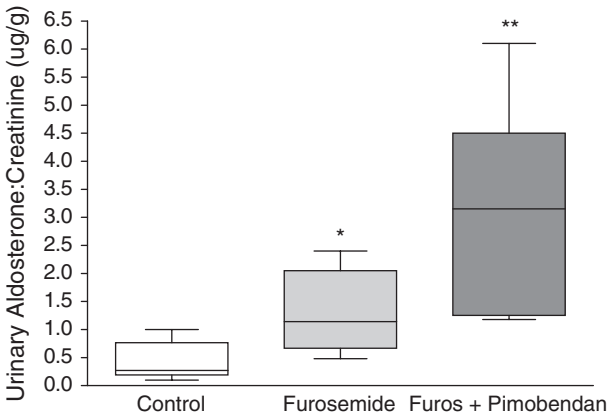

Fig 3. The urinary aldosterone to creatinine ratio (μg/g) at baseline, after 3 days of furosemide therapy (2 mg/kg BID), and after the addition of pimobendan (0.5 mg/kg BID) to furosemide treatment for 4 days. The box and whiskers plot demonstrates the median (line), 25 and 75% confidence intervals (box), and the range (bars). Compared to control values, there is activation of the circulating renin-angiotensin-aldosterone system with furosemide alone ($P < 0.008$*). The combination of furosemide and pimobendan increased the urinary aldosterone to creatinine ratio as compared with both the control situation and furosemide alone ($P < 0.004$**). Furos = furosemide.

Certain limitations to this study should be pointed out. The study used a small number of dogs, and the duration of the pharmacologic exposure was relatively brief. Doses of pimobendan were higher than the manufacturer's recommendation (0.25–0.6 mg/kg/day).[b] This dosage was to chosen to guarantee that, if an effect was present, it would be found, and is consistent with the dosage used in the authors' clinics in the treatment of refractory heart failure. The dosage chosen proved to be advantageous, as it showed that in the subacute setting, even at higher than recommended dosages in healthy dogs, pimobendan does not activate RAAS and does not suppress furosemide-activated RAAS. Another weakness in the study reported herein is that we evaluated the effects of the medications in healthy dogs, and dogs with heart disease or failure might respond differently. Finally, with the study design used, we were not able to ascertain the time of peak or the duration of RAAS activation with more chronic furosemide administration. Because of this, we cannot explain with certainty the apparent further increase in RAAS activation seen with pimobendan plus furosemide as compared with furosemide alone (Fig 3), and this should be evaluated further.

Despite these limitations, we conclude that pimobendan, when used as a single agent even at high dosages,

does not activate circulating RAAS, as indicated by the lack of an increase in average urinary aldosterone excretion in healthy dogs. RAAS activation is, however, observed in this population with both furosemide alone and combination of furosemide and pimobendan. Although these results should be confirmed in clinical patients, it appears that drugs, which blunt the RAAS (ACE-inhibitors, aldosterone receptor blockers, angiotensin II receptor blockers, and possibly β-blockers, alone or in combination) should be included in the pharmacopea for dogs receiving furosemide, with or without pimobendan.

Footnotes

[a] Cardell, Sharn Veterinary Inc, Tampa, FL
[b] Vetmedin, Boehringer Ingelheim, Berkshire, UK
[c] Package insert, Vetmedin
[d] Salix, Intervet Canada, Whitby, ON, Canada
[e] Esoterix Laboratories, Calabasas, CA

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References

1. Lovern CS, Swecker WS, Lee JC, Moon ML. Additive effects of sodium and chloride restricted diets and furosemide administration in healthy dogs. Am J Vet Res 2001;62:1783–1796.

2. Kammerl MC, Rolf MN, Schweda F, et al. Low sodium and furosemide-induced stimulation of the renin system in man mediated by cyclooxygenase2. Clin Pharm Ther 2001;70:468–473.

3. Atkins CE, Rausch WP, Gardner SY, et al. Effects 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.

4. Haggstrom J, Hansson K, Karlberg BE, et al. Effects of long term treatment with enalapril or hydralazine on the renin-angiotensin aldosterone system and fluid balance in dogs with naturally acquired mitral valve regurgitation. Am J Vet Res 1996;57:1645–1651.

5. Schrier RW, Abraham WT. Hormones and hemodynamics in heart failure. N Engl J Med 1999;341:577–585.

6. Weber KT. Aldosterone in congestive heart failure. N Engl J Med 2001;345:1689–1697.

7. Luis-Fuentes V. Use of pimobendan in the management of heart failure. Vet Clin North Am Small Anim Pract 2004;34:1145–1155.

8. Gordon S, Miller M, Saunders A. Pimobendan in heart failure therapy—A silver bullet? J Am Anim Hosp Assoc 2006;42:90–93.

9. Luis-Fuentes V, Cocoran B, French A, et al. A double-blind randomized placebo-controlled study of pimobendan in dogs with dilated cardiomyopathy. J Vet Int Med 2002;16:255–261.

10. Smith P, French AT, Van Israël N, et al. Long-term efficacy of pimobendan in slight-to-moderate heart failure caused by myxomatous mitral valve disease in dogs. A prospective, single-blind, parallel, 6-month comparison with ramipril. J Small Anim Pract 2005;46:121–130.

11. Häggström J, Boswood A, O'Grady M, et al. Effect of pimobendan or benazepril hydrochloride on survival times in dogs with congestive heart failure caused by naturally occurring myxomatous mitral valve disease: The QUEST study. J Vet Intern Med 2008;22:1124–1135.

12. Lombard CW, Jöns O, Bussadori CM. Veterinary study for the confirmation of pimobendan in canine endocardiosis. J Am Anim Hosp Assoc 2006;42:249–261.

13. Gardner SY, Atkins CE, Rausch WP, et al. Estimation of 24-h aldosterone secretion in the dog, using urine aldosterone : creatinine. J Vet Cardiol 2007;9:1–7.

14. Tidholm A, Haggstrom J, Hansson, et al. Effects of dilated cardiomyopathy on the rennin-angiotensin-aldosterone system, atrial natriuretic peptide, and thyroid hormone concentrations in dogs. Am J Vet Res 2001;62:961–967.