Assessment of diuretic effects and changes in plasma aldosterone concentration following oral administration of a single dose of furosemide or azosemide in healthy dogs

Yasutomo Hori, DVM; Akiko Katou, DVM; Mizuho Tsubaki, DVM; Kazutaka Kanai, DVM, PhD; Ruriko Nakao, DVM, PhD; Fumio Hoshi, DVM, PhD; Naoyuki Itoh, DVM, PhD; Sei-ichi Higuchi, DVM, PhD

Objective—To determine the diuretic effects and changes in plasma aldosterone concentration (PAC) following oral administration of a single dose of furosemide or azosemide in healthy dogs.

Animals—8 mixed-breed dogs.

Procedures—A single dose of furosemide (2 mg/kg), azosemide (1, 5, or 10 mg/kg), or placebo (bifidobacterium [1 mg/kg]) was administered orally (in random order at 7-day intervals) to each dog (5 treatments/dog). Urine and blood samples were collected before (2 hours after evacuation of the urinary bladder; baseline) and at intervals for 24 hours after drug treatment to assess urine volume and plasma and urine biochemical variables.

Results—Compared with baseline values, treatment with furosemide and azosemide (5 and 10 mg/kg) increased urine output for 1 to 2 hours and 2 to 4 hours, respectively. The 24-hour urine volume and urinary sodium excretion were significantly increased following furosemide and azosemide (5 and 10 mg/kg) treatments, compared with effects of placebo; these increases were dose dependent for azosemide, and increases were similar for furosemide and the 5 mg/kg dose of azosemide. Compared with other treatments, 24-hour urinary potassium excretion was significantly increased with azosemide at 10 mg/kg. Azosemide (5 and 10 mg/kg) significantly increased plasma total protein concentration and decreased plasma potassium concentration, compared with baseline values. Compared with the effect of placebo, PAC was significantly increased by furosemide and the 10 mg/kg dose of azosemide.

Conclusions and Clinical Relevance—In healthy dogs, a moderate dose of azosemide caused sufficient diuretic action and increased PAC to a lesser extent than furosemide. (Am J Vet Res 2008;69:1664–1669)

Congestive heart failure is a clinical syndrome caused by chronic heart disease and is characterized by sodium and water retention that results in edema. Loop diuretics (short- and long-acting agents) are commonly used in the management of CHF and are important for treatment of CHF-related signs. The loop diuretic furosemide is widely used to treat fluid retention, but its duration of action can be as short as a few hours and, as reported previously by our group, chronic administration of furosemide induces a decrease in the diuretic response in dogs. Diuretic resistance induced by chronic treatment with furosemide has been attributed in part to activation of the RAAS and induced expression of several transporter proteins in the kidneys. Furthermore, McCurley et al reported that furosemide accelerates the development of left ventricular systolic dysfunction and results in high serum aldosterone concentrations in pigs with experimentally induced tachycardia-related heart failure.

Azosemide (5-[4-chloro-5-sulfamoyl-2-thienylaminophenyl]tetrazole) is a long-acting loop diuretic that acts primarily at the thick ascending limb of the loop of Henle, where it blocks the active reabsorption of sodium and chloride ions. In humans, the diuretic effect of azosemide begins to increase 1 hour after oral administration and reaches a peak 2 to 3 hours later. The peak plasma concentration of the drug was detected 3 to 4 hours after administration. Previous studies have revealed that azosemide has clinical efficacy in humans with heart failure. In addition, it has been reported that, compared with the effects of furo-
Furosemide, azosemide improves ventricular fibrosis and decreases the mortality rate in rats with experimentally induced heart failure. However, basic information relating to the diuretic effect of azosemide in dogs is scarce. Furthermore, the clinically useful dosage of azosemide and diuretic response or changes in plasma aldosterone concentration associated with administration of the drug in dogs are unknown. The purpose of the study reported here was to determine the diuretic effects and changes in RAAS activity (eg, plasma aldosterone concentration) following oral administration of a single dose of furosemide or azosemide in healthy dogs.

Materials and Methods

Animals—Eight mixed-breed dogs of both sexes were used in the study. The dogs were 2 to 8 years old and weighed 8 to 18 kg. All dogs were housed individually in cages. The dogs were given commercial dry food and had free access to water. Four dogs had been used in a previous study performed at least 1 year earlier. The Guidelines for Institutional Laboratory Animal Care and Use of the School of Veterinary Medicine at Kitasato University were followed during the study.

Experimental protocol—In a crossover study, dogs were initially randomized to receive placebo, furosemide, or azosemide. Treatments were administered orally and included placebo (bifidobacterium [1 mg/kg]), furosemide (2 mg/kg), or azosemide (1, 5, or 10 mg/kg). Each drug was given randomly, and the interval between treatments was at least 7 days. After each washout period, each dog was then randomly assigned to receive one of the remaining treatments. This continued until each dog had received all 5 treatments. Prior to administration of a treatment, a 6- to 8-F Foley catheter was placed in the urinary bladder to collect urine samples. The catheter remained inserted in the urinary bladder during the 24-hour posttreatment period. Two hours after complete evacuation of the urinary bladder and before administration of the assigned treatment, blood (5 mL) and urine samples were collected to provide baseline data. The diuretic or placebo was then administered orally and blood (5 mL) and urine samples were collected 1, 2, 4, 6, 8, 12, and 24 hours after administration. The dogs were not fed on a day of the sample collection, but they had free access to water. The catheter was removed from the urinary bladder after the final urine samples were collected at the 24-hour time point.

Plasma and urine analyses—Each blood sample was obtained from a cephalic vein into tubes containing heparin for hematologic and plasma biochemical analyses and into tubes containing EDTA for determination of PAC. The plasma was separated via centrifugation at 1,500 g for 10 minutes at 4°C and was stored in tubes at –80°C. The collected urine samples were centrifuged at 1,500 g for 5 minutes at 4°C, and the supernatant was stored in tubes at –80°C. Plasma protein concentration was measured via refractometry. The Hct was determined by use of the microhematocrit method. Plasma BUN and creatinine concentrations (mg/dL) were measured by use of an autoanalyzer. Plasma sodium, potassium, and chloride concentrations (mEq/L) were measured by use of an electrolyte analyzer. Urine sodium and potassium concentrations were measured by use of an electrolyte analyzer, and the 24-hour urinary sodium and potassium excretions were calculated. Plasma aldosterone concentration was determined via radioimmunoassay. The urine output (mL/kg/h), 24-hour urine volume, and USG were immediately measured after collection of urine at each time point.

Statistical analysis—Data were reported as mean values ± SD. Urine output and USG at the various time points after administration of each drug were compared with the baseline values by use of a 1-factor repeated-measures ANOVA. A 2-way ANOVA was used to compare the time course of changes in urine output and USG among treatments. A 1-way ANOVA was used to analyze the 24-hour urine volume and 24-hour urinary sodium and potassium excretions among treatments. The significance of the differences among the mean values at baseline and at each time point for each treatment was evaluated by use of a Tukey multiple comparison test. The differences in plasma BUN, creatinine, and electrolyte concentrations at baseline and 24 hours after administration of each drug were analyzed by use of a paired t-test. The Kruskal-Wallis test was used to analyze PAC among the treatments, and a Tukey test was used for post hoc analysis. A value of P < 0.05 was considered significant.

Results

Among the study dogs, administration of 2 mg of furosemide/kg induced an increase in urine output, compared with the baseline value, and peak urine output occurred 2 hours after administration (Figure 1). The USG was significantly decreased following treatment with furosemide, compared with the baseline value, and the decrease persisted for 1 to 8 hours (Figure 2). Compared with placebo, furosemide significantly (P < 0.001) increased urine output and decreased USG.

Figure 1—Mean ± SD urine output in 8 healthy dogs determined before (time 0 hours; baseline) and at intervals after oral administration of a single dose of furosemide (2 mg/kg), azosemide (1, 5, or 10 mg/kg), or placebo (bifidobacterium [1 mg/kg]) in a crossover study. Treatments were administered in random order at 7-day intervals to each dog (6 treatments/dog). Baseline values were evaluated 2 hours after complete evacuation of the urinary bladder but prior to administration of any treatment. *Value is significantly (P < 0.001) different from the baseline value.
Compared with findings after treatment with placebo, urine output was not changed by administration of azosemide at a dose of 1 mg/kg, whereas it was increased significantly (P < 0.001) following administration of azosemide at doses of 5 and 10 mg/kg (Figure 1). Compared with baseline value, urine output was increased significantly by treatment with the 5 mg/kg dose of azosemide at 2 to 4 hours; at 2 and 4 hours, the increase in urine output following treatment with the 10 mg/kg dose of azosemide was comparatively greater. The change in urine output over time was significantly (P = 0.01) greater for azosemide at the 10 mg/kg dose, compared with the effect of the 5 mg/kg dose. Compared with placebo values, USG was decreased significantly (P < 0.001) after administration of azosemide at doses of 5 and 10 mg/kg (Figure 2). Compared with baseline, the significant decrease in USG persisted from 2 to 6 hours for the 5 mg/kg dose of azosemide and from 1 to 8 hours for the 10 mg/kg dose of azosemide. The decrease in USG was significantly (P < 0.05) greater with azosemide at a dose of 10 mg/kg than the decrease achieved with furosemide.

Compared with placebo and azosemide administered at a dose of 1 mg/kg, treatments with furosemide and the 5 and 10 mg/kg doses of azosemide significantly increased the 24-hour urine volume; azosemide at 10 mg/kg had a significantly greater effect than the drug at 5 mg/kg (Figure 3). The 24-hour urinary sodium excretion was significantly higher following administration of furosemide and 5 or 10 mg/kg doses of azosemide than it was following administration of placebo or the 1 mg/kg dose of azosemide (Figure 4). The 24-hour urinary sodium excretion after treatment with 10 mg of azosemide/kg was significantly higher than the values determined after treatment with fu-
Azosemide or 5 mg of azosemide/kg. The 24-hour urinary potassium excretion was significantly increased following administration of the 10 mg/kg dose of azosemide, compared with the values following administration of all other treatments.

Compared with baseline findings, there were no significant changes in body mass, Hct, or plasma sodium, BUN, or creatinine concentrations in the study dogs at 24 hours after administration of any treatment (Table 1). Furosemide and azosemide administered at doses of 5 and 10 mg/kg significantly increased plasma protein concentration at the 24-hour time point, compared with baseline values. Plasma potassium concentration was significantly decreased from the baseline value after treatment with 5 and 10 mg of azosemide/kg.

Compared with the placebo treatment value, PAC was significantly increased 24 hours after administration of furosemide (5-fold increase) and the 10 mg/kg dose of azosemide (7-fold increase) but not after administration of the 5 mg/kg dose of azosemide (Figure 5).

Discussion

Loop diuretics are powerful natriuretic agents used in the management of CHF in various species. The loop diuretic furosemide is widely used in clinical settings because of its rapid action and strong natriuretic effect. In the present study in healthy dogs, an increase in urine output was evident at 1 hour after oral administration of furosemide (2 mg/kg); the peak response was detected at 2 hours after administration, but the response was transient. Previously, we reported that furosemide evokes rapid and short-action diuretic effects in healthy dogs, which is consistent with the findings of the present study. In addition, compared with the placebo treatment value, there was a 5-fold increase in PAC as a result of the administration of furosemide. Previous studies have revealed that treatment with furosemide activates the RAAS in humans and dogs. The data obtained in our study suggest that furosemide induces a rapid diuretic response and the reduction in total body water may activate the RAAS.

The results of the present study indicated that a single dose of azosemide at 5 or 10 mg/kg significantly increased urine output in healthy dogs. The diuretic action of azosemide at a dose of 5 mg/kg persisted for 4 hours and that of azosemide at a dose of 10 mg/kg reached a peak at 2 hours and persisted for another 2 hours in healthy dogs. These data are consistent with findings of a study in humans, in which urine volume was increased (compared with the pretreatment value) at 1 hour after oral administration of azosemide, and this increase continued for 5 hours (peaking at 2 to 4 hours).

The difference in the duration of the diuretic phases between furosemide and azosemide in humans and

Table 1—Body weight, Hct, and plasma concentrations of several variables (mean ± SD values) in 8 healthy dogs determined before (baseline) and at 24 hours after oral administration of a single dose of furosemide (2 mg/kg), azosemide (1, 5, or 10 mg/kg), or placebo (bifidobacterium [1 mg/kg]) in a crossover study. Treatments were administered in random order at 7-day intervals to each dog (5 treatments/dog). Baseline values were evaluated 2 hours after complete evacuation of the urinary bladder but prior to administration of any treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time point</th>
<th>Weight (kg)</th>
<th>Hct (%)</th>
<th>PP (g/dL)</th>
<th>Na (mEq/L)</th>
<th>K (mEq/L)</th>
<th>Cl (mEq/L)</th>
<th>BUN (mg/dL)</th>
<th>Creatinine (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>Baseline</td>
<td>12.3 ± 2.4</td>
<td>42 ± 5</td>
<td>6.2 ± 0.6</td>
<td>158 ± 9</td>
<td>5.0 ± 0.5</td>
<td>120 ± 7</td>
<td>22 ± 6</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>Furosemide</td>
<td>Baseline</td>
<td>11.8 ± 2.0</td>
<td>41 ± 2</td>
<td>6.5 ± 0.9</td>
<td>161 ± 2</td>
<td>4.8 ± 0.3</td>
<td>124 ± 2</td>
<td>21 ± 7</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>Azosemide (1 mg/kg)</td>
<td>24 hours</td>
<td>10.9 ± 2.7</td>
<td>42 ± 5</td>
<td>6.5 ± 0.3</td>
<td>153 ± 3</td>
<td>4.5 ± 0.3</td>
<td>121 ± 5</td>
<td>24 ± 4</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>Azosemide (5 mg/kg)</td>
<td>24 hours</td>
<td>10.7 ± 2.6</td>
<td>45 ± 5</td>
<td>6.8 ± 0.4*</td>
<td>152 ± 5</td>
<td>4.3 ± 0.4</td>
<td>121 ± 8</td>
<td>22 ± 7</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>Azosemide (10 mg/kg)</td>
<td>24 hours</td>
<td>10.5 ± 3.0</td>
<td>40 ± 7</td>
<td>6.2 ± 0.6</td>
<td>156 ± 7</td>
<td>4.8 ± 0.4</td>
<td>120 ± 4</td>
<td>25 ± 6</td>
<td>0.9 ± 0.1</td>
</tr>
</tbody>
</table>

*Value is significantly (P < 0.05) different from the baseline value. †Value is significantly (P = 0.01) different from the baseline value. ‡Value is significantly (P < 0.001) different from the baseline value.

PP = Plasma protein.
dogs was previously reported.\textsuperscript{10,14,16,18,21} Tomiyama et al\textsuperscript{13} reported that the 24-hour urinary sodium excretion in patients with CHF was similar during treatment with 40 mg of furosemide and with 60 mg of azosemide but was significantly greater in the first 2 hours after furosemide administration. This difference was explained by the fact that the estimated extent of absolute oral bioavailability of azosemide is relatively low in humans (10% to 20%), as it is in dogs (23.8% to 47.3%).\textsuperscript{10,16,21} In addition, the elimination half-life of azosemide (2 to 2.5 hours) is somewhat longer than that of other loop diuretics.\textsuperscript{10,22} In 1 study,\textsuperscript{21} azosemide (60 mg, q 24 h) did not appear to accumulate in dogs because the plasma concentrations of the diuretic, as well as the area under the curve, were highly similar at days 1 and 21 of treatment. When 40 mg of azosemide was administered as a once-daily oral treatment for 5 consecutive days to healthy humans, urine output increased (from the baseline value) at 1 hour after administration and continued for 5 hours on day 1. By day 5, the increase in urine output continued for approximately 9 hours.\textsuperscript{15} These results indicate a longer diuretic phase associated with oral administration of azosemide than with oral administration of furosemide in healthy dogs and humans.

Although the 24-hour urine volume in healthy dogs increased similarly after administration of furosemide (2 mg/kg) and azosemide (5 mg/kg) in our study, furosemide (2 mg/kg) was associated with a 5-fold increase in PAC, compared with the placebo treatment value, whereas azosemide (5 mg/kg) was associated with no significant change in PAC. On the other hand, azosemide at a dose of 10 mg/kg significantly increased both the 24-hour urine volume and PAC. The difference in the response to azosemide is mainly a result of the dose dependency of the diuretic and natriuretic actions of this agent. It is known that long-term administration of furosemide induces a decrease in diuretic response and an increase in PAC in healthy dogs.\textsuperscript{2} In studies\textsuperscript{14,18} of patients with CHF, plasma concentrations of active renin and norepinephrine were significantly higher after treatment with furosemide than values after treatment with azosemide, and furosemide had a greater influence on heart rate variability than azosemide. The results of the present study indicated that the increase in PAC associated with the short-acting loop diuretic furosemide was marked, whereas that associated with the long-acting loop diuretic azosemide administered at a dose of 5 mg/kg was less dramatic.

A recent retrospective study\textsuperscript{23} in humans with left ventricular dysfunction revealed that the administration of non–potassium-sparing diuretics (including furosemide) was associated with increased risk of hospitalization for progression of CHF, increased risk of death from progressive CHF, and increased cardiovascular and all-cause mortality rates, compared with the effects of administration of no diuretic agent or potassium-sparing diuretics (including spironolactone). Similarly, a randomized aldactone evaluation study\textsuperscript{24} revealed that treatment with spironolactone reduces the risk of death, reduces hospitalization rates, and improves symptoms in humans with CHF largely as a result of interference with the neurohumoral system. The results of these human clinical studies suggest that diuretics that do not activate the RAAS improve prognosis in patients with CHF. In the present study in healthy dogs, a single moderate dose of azosemide induced sufficient diuretic effect with weak RAAS activation. Therefore, long-term administration of azosemide in dogs in clinical settings may be attractive. Further studies are warranted to address the long-term response to diuretics (including diuretic resistance and prognosis) in dogs with heart disease.

The adverse effects of loop diuretics include natriuresis, kaliuresis, and hypokalemia; those drugs may also induce dehydration, hypokalemia, and azotemia.\textsuperscript{12,22} In the present study, azosemide at a dose of 10 mg/kg caused a significant increase in 24-hour urinary potassium excretion in healthy dogs. In addition, azosemide induced an increase in plasma protein concentration and a decrease in plasma potassium concentration. After 26 consecutive weeks of oral administration of azosemide (80 mg/kg/d) in Beagles, adverse effects included loss of appetite, decrease in weight gain, inhibition of voluntary movement, weakness, and sialis.\textsuperscript{26} Furthermore, repeated infusion of azosemide in humans resulted in a progressive decrease in the natriuretic effect, which was associated with an increase in plasma renin activity.\textsuperscript{27}

The present study was undertaken to investigate the diuretic effects of long-acting loop diuretics and their influence on PAC in healthy dogs. Although the number of dogs included in the study was small, a controlled study was performed. When coadministered with an ACE inhibitor, digoxin, or a β-adrenergic receptor antagonist, diuretics might have effects in dogs that are different from those detected in our study. In addition, we cannot exclude the possibility that chronic heart disease may lead to different responses. The 24-hour urine volume increased similarly with single doses of azosemide (5 mg/kg) and furosemide (2 mg/kg); in this experimental setting, the data suggest that a moderate dose of azosemide causes mild and long-acting effects with minimal activation of the RAAs, yet results in adequate diuresis. Further studies to investigate the clinical usefulness of azosemide in dogs with heart disease are warranted.

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\textbf{References}
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