Comparative Adverse Cardiac Effects of Pimobendan and Benazepril Monotherapy in Dogs with Mild Degenerative Mitral Valve Disease: A Prospective, Controlled, Blinded, and Randomized Study

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Background: Pimobendan (PIMO) is an inodilator that may have some beneficial effects in canine degenerative mitral valve disease (MVD). However, little information is available about its cardiac effects in dogs without systolic myocardial dysfunction.

Hypothesis: Compared to benazepril (BNZ), an angiotensin-converting enzyme inhibitor, PIMO may worsen valve regurgitation in early canine MVD.

Animals: Twelve Beagles with asymptomatic MVD were randomized into 2 groups (n = 6) receiving BNZ or PIMO at dosages of 0.25 mg/kg PO q24h and q12h respectively, for 512 days.

Methods: The study followed a blinded, randomized, prospective, and parallel group design. After day 512, the dogs were necropsied, and cardiac histopathology was performed in a blinded manner.

Results: A significant treatment effect was observed as soon as day 15 with increased systolic function in the PIMO group by comparison to baseline value as assessed by fractional shortening (P < .0001) and tissue Doppler variables (P = .001). Concurrently, the maximum area and peak velocity of the regurgitant jet signal increased (P < .001), whereas these variables remained stable in the BNZ group. Histologic grades of mitral valve lesions were more severe in the PIMO group than in the BNZ group.

Conclusions and Clinical Importance: PIMO has adverse cardiac functional and morphologic effects in dogs with asymptomatic MVD. Additional investigation in dogs with symptomatic MVD is now warranted.

Key words: Angiotensin-converting enzyme inhibitor; Inotrope; Phosphodiesterase III inhibitor.

Medications manage heart failure requires chronic use of effective and safe drugs. Angiotensin-converting enzyme inhibitors (ACEI) represent one of the most commonly used categories of drugs in human and canine cardiology. During the last 10 years, the efficacy and long-term tolerability of benazepril (BNZ) and other ACEI have been demonstrated convincingly in dogs by clinical trials involving large numbers of animals, especially those affected by degenerative mitral valve disease (MVD). Several prospective, double-blinded, multicentric, and randomized studies have shown that ACEI improve quality of life, increase exercise tolerance, and extend life expectancy in dogs with naturally acquired New York Heart Association class II-IV heart failure. 

Kvart et al also showed that long-term enalapril monotherapy was well tolerated in Cavalier King Charles Spaniels with asymptomatic MVD.

Pimobendan (PIMO) is an orally active drug combining calcium-sensitizing properties with cyclic AMP phosphodiesterase (PDE) III inhibition. These actions result in positive inotropic and vasodilatory effects, which may be beneficial in dogs with left ventricular systolic failure, as demonstrated in Doberman Pinschers with dilated cardiomyopathy. PIMO also is registered in many countries for use in canine congestive heart failure due to MVD, and some recent studies suggest that it may be beneficial in mild to moderate stages of the disease. On the other hand, previous experimental data demonstrated that PIMO has potential adverse effects and that valvular and parietal endocardial jet lesions could be induced in healthy dogs, even in the absence of previous valvular disease, after 4 weeks of repeated PIMO administration at dosages close to therapeutic ones. Moreover, individual reports of dogs chronically treated with PIMO recently have described adverse cardiac effects that were, at least in part, reversed after cessation of PIMO administration. All of these findings, together with current views about PDE III inhibitors in human medicine, have raised an important question: what are the potential cardiotoxic effects of chronic treatment with PIMO in dogs without systolic myocardial dysfunction?

The aims of this prospective, blinded, and randomized study therefore were (1) to compare the cardiac pharmacodynamics of long-term monotherapy with PIMO or BNZ in dogs with spontaneous asymptomatic MVD using conventional echo-Doppler and tissue Doppler imaging (TDI) techniques and (2) to determine whether the chronic use of PIMO or BNZ at the recommended dosages could produce cardiac lesions. Dogs with stage I MVD and mild mitral regurgitation were included in this study to limit any potential...
interaction between the spontaneous worsening of the valvular disease and any adverse cardiac effects actually induced by the medical treatments, ie, to assess the intrinsic cardiac effects of each drug. Because they were euthanized at the end of the study period, the animals involved in the present study could not be client-owned dogs. Therefore we decided to select dogs with stage I MVD from animals belonging to a French breeder who owned a large population of Beagle dogs.

Materials and Methods

Animals

The study was conducted in compliance with the Procedures and Principles of Good Clinical Practice. The protocol was approved by the French Ministry of Agriculture and was performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Twelve female Beagle dogs with asymptomatic (NYHA class I) MVD (age, 6.8 ± 2.1 years; range, 4.3–9.3 years; weight, 14.2 ± 1.9 kg; range, 11.5–16.7 kg) were selected from a population of 450 adult Beagle dogs from 42 different families. The inclusion criteria were the following: (1) left apical systolic heart murmur of low grade (I or II/VI); (2) echocardiographic and Doppler signs of MVD, including irregular and thickened mitral valve leaflets observed on the right parasternal 4-chamber view associated with a color-flow jet of mitral insufficiency in the left atrium on the left parasternal 4-chamber view; (3) left atrial and ventricular diameters within the normal ranges, with no decrease in systolic function confirmed by normal fractional shortening (%FS) and normal systolic myocardial velocities assessed by tissue Doppler imaging. Animals were housed in collective enclosures with access to outer enclosures. Fresh water and commercial low fat food were supplied daily.

Treatment, Blinding Procedure, and Experimental Design

The 12 Beagle dogs were randomized into 2 groups (n = 6) to receive BNZ or PIMO PO at dosages of 0.25 mg/kg q24h (BNZ) and q12h (PIMO) for 512 days. According to the manufacturer’s recommendations, PIMO was administered at least 1 hour before feeding. Because BNZ and PIMO dosage regimes and oral formulations were different, blinding was maintained by the double-investigator technique: 1 investigator (VC) was responsible for clinical and imaging assessments, and 2 other investigators (VG, CCS) were responsible for treatment administration. Before the start of treatment (day 0) and then at 15, 49, 124, 206, 322, 414, and 512 days after therapy initiation, all dogs underwent cardiovascular examination with heart murmur grading, arterial blood pressure measurement using the oscillometric method, standard echo-Doppler examination, two-dimensional (2D) color TDI, and blood analysis (CBC and plasma chemistry including alanine aminotransferase, alkaline phosphatase, total protein, urea, creatinine, and glucose). Moreover, at the end of the study period, glomerular filtration rate (GFR) was assessed in all dogs using the plasma exogenous creatinine clearance test as previously validated by our group because of potential renal safety issues due to the observed hemodynamic effects.

To perform an adequate analysis of imaging data, all variations after drug administration were interpreted according to the involved investigator’s variability, which has already been published for all of the conventional echocardiographic and TDI variables except for the maximal thickness of the mitral valve leaflet. A validation protocol was performed during the study period to assess the intraobserver variability of the latter variable and of all the conventional Doppler variables (see validation of Conventional Echo-Doppler Variables section).

Conventional Echocardiography and Doppler Examination

Standard transthoracic echocardiography with continuous ECG monitoring was performed on awake dogs using an ultrasound unit equipped with 2.2–3.5 MHz and 5.5–7.5 MHz phased-array transducers as previously validated at the cardiology unit. The left ventricular myocardial walls and diameters were measured, as were fractional shortening (%FS) using the 2D-guided M-mode and maximal thickness of the anterior mitral valve leaflet using the right parasternal 4-chamber view. Left atrial diameter/aortic diameter ratio (LA/Ao) was also assessed. Mitral regurgitant jet area was assessed by the Doppler color flow mapping method as previously described, and the maximum area of the regurgitant jet signal/left atrium area ratio (ARJ/LAA) was calculated. The peak velocity and the velocity time integral of the regurgitant jet were measured by continuous-wave Doppler using the left apical 4-chamber view. Last, aortic stroke volume was assessed by pulsed wave Doppler trace of the aortic flow velocity by multiplying the aortic area by the velocity time integral using the left apical 5-chamber view.

Validation of Conventional Echo-Doppler Variables

In parallel to the reported study, intraday and interday variabilities of the above Doppler variables and of the maximal thickness of the anterior mitral valve leaflet were determined by performing 36 Doppler examinations on 6 nontreated dogs affected by asymptomatic MVD (age, 11.5 ± 2.4 years; range, 9–15 years; weight, 7.6 ± 4.0 kg; range, 3.1–14.4 kg) on 4 different days during a 2-week period: 3 Poodles, 1 Beagle, 1 Yorkshire Terrier, and 1 Shih-tzu. On a given day, 3 dogs were examined at 3 nonconsecutive times. Each variable was measured 3 times on 3 consecutive cardiac cycles using the same frame, and the mean values were used to determine the intra- and interday variabilities. Results are reported in Table 1.

2D Color TDI Examinations

As previously described and validated, 2D color TDI examinations were performed in awake dogs with continuous ECG monitoring and with the same ultrasound unit as for the conventional echocardiographic examinations using image management software. Systolic LVFW velocities resulting from radial contraction were measured in an endocardial and epicardial segment, and the corresponding myocardial velocity gradient (MVG), defined as the difference between endocardial and epicardial velocities, was calculated. Moreover, systolic left ventricular free wall (LVFW) velocities resulting from the longitudinal contraction were measured in a basal segment.

Postmortem Examination and Histopathology

The dogs were euthanized on day 512 by an overdose of IV sodium pentobarbital. Histopathologic examinations were performed by a board-certified veterinary pathologist in a blinded fashion (ie, the observer did not know the treatment group). A second pathologist (a diplomat of the French Veterinary Histopathological Board) who also did not know the treatment group was asked to examine the sections to verify the scores of the first pathologist because of the presence of specific lesions. All cardiac specimens were fixed in 10% formalin. Tissues (mitral and tricuspid valves, right and left ventricles, auricles, and septum) were embedded in paraffin, sectioned at 4 µm, and stained with hematoxylin and eosin and hematoxylin, eosin, and safranin. In addition, hemosiderin and glycosaminoglycan deposits in the
Table 1. Intra- and inter-day variability of conventional echo-Doppler variables assessed in this study. The repeatability and the reproducibility of the other conventional echocardiographic variables have been published previously.19

<table>
<thead>
<tr>
<th>Echocardiographic variable</th>
<th>Intra-Day</th>
<th>Inter-Day</th>
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<tbody>
<tr>
<td></td>
<td>SD</td>
<td>CV (%)</td>
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<tr>
<td>Maximal thickness of the anterior mitral valve leaflet (mm)</td>
<td>0.5</td>
<td>6.9</td>
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<tr>
<td>Doppler variables</td>
<td></td>
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<tr>
<td>ARJ/LAA ratio (%)</td>
<td>3.9</td>
<td>7.1</td>
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<tr>
<td>Peak velocity of the mitral regurgitant jet (m/s)</td>
<td>0.2</td>
<td>2.8</td>
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<tr>
<td>Velocity time integral of the mitral regurgitant jet (mm)</td>
<td>91</td>
<td>8.8</td>
</tr>
<tr>
<td>Aortic systolic peak velocity (m/s)</td>
<td>0.1</td>
<td>4.2</td>
</tr>
<tr>
<td>Aortic stroke volume (mL)</td>
<td>2.7</td>
<td>16.5</td>
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</table>

ARJ/LAA, maximum area of the regurgitant jet signal/Left atrium area; CV, coefficients of variation; SD, standard deviation.

Statistical Analysis

Data are expressed as mean ± SD. Statistical analyses were performed for quantitative variables (eg, weight, blood pressure, heart rate, conventional echo-Doppler and TDI variables, and GFR) by computer software.2 Treatment comparability at baseline was assessed using Student’s t-tests and Fisher’s exact probability tests for quantitative variables and qualitative variables, respectively. The experimental design was a repeated measures (factorial split-plot) design. The difference of observed values with the baseline value was analyzed using a model including the treatment (BNZ or PIMO), the treatment period (day of investigation), an interaction between treatment and period as fixed effects factors, and the animal (dog) as a random effects factor. When the interaction between treatment and period was significant, a multiple comparison was performed using Bonferroni adjustment. When the interaction was not significant but the treatment effect was significant, the difference between treatments was significant for the whole study period, ie, from day 15 to day 512.

ARJ/LAA, maximum area of the regurgitant jet signal/Left atrium area; CV, coefficients of variation; SD, standard deviation.

Table 2. Means ± SD of body weight, heart rate assessed by ECG, systolic, and diastolic blood pressure in the pimobendan (PIMO, n = 6) and benazepril (BNZ, n = 6) groups before initiation of therapy (day 0) and at day 15, 49, 124, 206, 322, 414, and 512. Dogs from the BNZ and the PIMO groups received 0.25 mg/kg PO q24h of benazepril and 0.25 mg/kg PO q12h of pimobendan, respectively.

<table>
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<tr>
<th></th>
<th>Day 0</th>
<th>Day 15</th>
<th>Day 49</th>
<th>Day 124</th>
<th>Day 206</th>
<th>Day 322</th>
<th>Day 414</th>
<th>Day 512</th>
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<tr>
<td>Body weight (kg)</td>
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<tr>
<td>PIMO group</td>
<td>13.5 ± 1.6</td>
<td>13.4 ± 1.3</td>
<td>13.0 ± 1.6</td>
<td>13.2 ± 1.6</td>
<td>13.4 ± 1.3</td>
<td>14.2 ± 1.5</td>
<td>12.0 ± 1.1</td>
<td>10.6 ± 0.9</td>
</tr>
<tr>
<td>BNZ group</td>
<td>14.9 ± 2.0</td>
<td>15.4 ± 2.2</td>
<td>14.7 ± 2.1</td>
<td>14.8 ± 1.9</td>
<td>14.3 ± 2.0</td>
<td>15.3 ± 2.0</td>
<td>13.3 ± 2.0</td>
<td>13.2 ± 1.9</td>
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<td>Heart rate (beats/min)</td>
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<tr>
<td>PIMO group</td>
<td>103 ± 12</td>
<td>112 ± 12</td>
<td>103 ± 9</td>
<td>106 ± 19</td>
<td>118 ± 7</td>
<td>118 ± 24</td>
<td>112 ± 15</td>
<td>110 ± 5</td>
</tr>
<tr>
<td>BNZ group</td>
<td>113 ± 15</td>
<td>123 ± 15</td>
<td>109 ± 25</td>
<td>123 ± 15</td>
<td>115 ± 16</td>
<td>125 ± 15</td>
<td>130 ± 13</td>
<td>112 ± 24</td>
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<td>Systolic blood pressure (mmHg)</td>
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<tr>
<td>PIMO group</td>
<td>133 ± 18</td>
<td>128 ± 21</td>
<td>126 ± 9</td>
<td>131 ± 10</td>
<td>136 ± 15</td>
<td>141 ± 7</td>
<td>144 ± 9</td>
<td>142 ± 12</td>
</tr>
<tr>
<td>BNZ group</td>
<td>144 ± 11</td>
<td>134 ± 22</td>
<td>131 ± 16</td>
<td>126 ± 12</td>
<td>138 ± 10</td>
<td>143 ± 7</td>
<td>143 ± 12</td>
<td>134 ± 13</td>
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<tr>
<td>Diastolic blood pressure (mmHg)</td>
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<tr>
<td>PIMO group</td>
<td>70 ± 14</td>
<td>87 ± 18</td>
<td>79 ± 12</td>
<td>86 ± 8</td>
<td>83 ± 10</td>
<td>93 ± 6</td>
<td>90 ± 5</td>
<td>82 ± 9</td>
</tr>
<tr>
<td>BNZ group</td>
<td>73 ± 11</td>
<td>86 ± 22</td>
<td>85 ± 10</td>
<td>85 ± 14</td>
<td>92 ± 6</td>
<td>89 ± 7</td>
<td>93 ± 6</td>
<td>75 ± 11</td>
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</tbody>
</table>

Heart murmur grades between PIMO and BNZ groups were compared using a Fisher’s exact test.

The following linear model was used for the intraday and interday variability analysis:20

\[ Y_{ijk} = \mu + d_{iy} + d_{jy} + (day \times dog)_y + e_{ijk} \]

where \( Y_{ijk} \) is the \( k \)th value measured for dog \( j \) on day \( i \), \( \mu \) is the general mean, \( d_{iy} \) is the differential effect of dog \( j \), \( d_{jy} \) is the differential effect of dog \( j \), \( (day \times dog)_y \) is the interaction term between day and dog, and \( e_{ijk} \) is the model error. The standard deviation (SD) of repeatability was estimated as the residual SD of the model and the SD of reproducibility as the SD of the differential effect of day. The corresponding coefficients of variation (CV) were determined by dividing each SD by the mean. \( P \) values < .05 were considered statistically significant.

Results

Treatment groups were comparable at baseline with respect to all parameters (eg, weight, blood pressure, heart rate, CBC, plasma chemistry, conventional echo-Doppler variables, and TDI variables).
Treatment Effects on Body Weight, Blood Analyses, and GFR

Body weight, plasma CBC, and clinical chemistry parameters remained stable throughout the study period in all dogs (Table 2). Conversely, GFR was significantly lower \((P < .05)\) at day 512 in the PIMO group \((4.2 \pm 0.5 \text{ mL/min/kg})\) as compared with the BNZ group \((4.8 \pm 0.2 \text{ mL/min/kg})\).

Treatment Effects on ECG, Arterial Blood Pressure, and Heart Murmur Grade

Heart rate and arterial blood pressure remained stable throughout the study period (Table 2). As shown in Figure 1, at day 0, all dogs showed a grade I heart murmur except 1 dog with a grade II murmur in the BNZ group. Heart murmur grade was significantly higher \((P < .05)\) in the PIMO group on day 124 by comparison with the BNZ group and remained higher throughout the remaining study period. At day 512, heart murmur was increased by 2 grades on average in the PIMO group.

Treatment Effects on Systolic Myocardial Function and Heart Morphology

All of the conventional echocardiographic and TDI variables remained unchanged throughout the 512-day study period in the BNZ group (Figs 2, 3; Table 3). Conversely, the %FS beginning at day 15 was significantly higher \((P < .0001)\) than baseline in the PIMO group; this was due to a decrease \((P = .001)\) of the left ventricular systolic diameter with an unchanged di-
Table 3. Means ± SD of systolic tissue Doppler imaging variables in the pimobendan (PIMO, n = 6) and benazepril (BNZ, n = 6) group before initiation of therapy (day 0) and at day 15, 49, 124, 206, 322, and 512. Dogs from the BNZ and the PIMO groups received 0.25 mg/kg PO q24h of benazepril and 0.25 PO mg/kg q12h of pimobendan, respectively.

<table>
<thead>
<tr>
<th>Systolic radial motion</th>
<th>Day 0</th>
<th>Day 15</th>
<th>Day 49</th>
<th>Day 124</th>
<th>Day 206</th>
<th>Day 322</th>
<th>Day 414</th>
<th>Day 512</th>
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<tbody>
<tr>
<td>S wave in subendocardium (cm/s)</td>
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<tr>
<td>PIMO group**</td>
<td>5.1 ± 1.7</td>
<td>7.4 ± 1.8</td>
<td>6.5 ± 1.7</td>
<td>6.9 ± 1.6</td>
<td>7.5 ± 1.4</td>
<td>6.9 ± 1.9</td>
<td>6.2 ± 2.3</td>
<td>6.3 ± 1.5</td>
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<tr>
<td>BNZ group</td>
<td>5.4 ± 1.1</td>
<td>5.3 ± 0.7</td>
<td>4.8 ± 0.9</td>
<td>4.9 ± 1.0</td>
<td>5.2 ± 0.4</td>
<td>5.3 ± 0.9</td>
<td>5.1 ± 0.5</td>
<td>4.9 ± 0.3</td>
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<tr>
<td>S wave in subepicardium (cm/s)</td>
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<tr>
<td>PIMO group*</td>
<td>2.5 ± 1.2</td>
<td>3.7 ± 1.1</td>
<td>3.3 ± 2.0</td>
<td>3.2 ± 1.0</td>
<td>4.1 ± 1.0</td>
<td>3.7 ± 0.9</td>
<td>3.3 ± 1.5</td>
<td>3.1 ± 0.5</td>
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<tr>
<td>BNZ group</td>
<td>3.1 ± 0.9</td>
<td>2.8 ± 0.9</td>
<td>2.3 ± 0.4</td>
<td>2.6 ± 0.9</td>
<td>2.9 ± 0.4</td>
<td>2.8 ± 0.9</td>
<td>2.8 ± 0.6</td>
<td>2.3 ± 0.7</td>
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<tr>
<td>Gradient between subendocardium and subepicardium (cm/s)</td>
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<tr>
<td>PIMO group***</td>
<td>2.6 ± 0.9</td>
<td>3.8 ± 1.2</td>
<td>3.3 ± 0.5</td>
<td>3.7 ± 1.1</td>
<td>3.4 ± 0.5</td>
<td>3.2 ± 1.2</td>
<td>2.9 ± 1.1</td>
<td>3.3 ± 1.2</td>
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<tr>
<td>BNZ group</td>
<td>2.3 ± 0.6</td>
<td>2.5 ± 0.5</td>
<td>2.4 ± 0.6</td>
<td>2.3 ± 0.6</td>
<td>2.3 ± 0.4</td>
<td>2.5 ± 0.3</td>
<td>2.3 ± 0.7</td>
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</table>

<table>
<thead>
<tr>
<th>Systolic longitudinal motion</th>
<th>Day 0</th>
<th>Day 15</th>
<th>Day 49</th>
<th>Day 124</th>
<th>Day 206</th>
<th>Day 322</th>
<th>Day 414</th>
<th>Day 512</th>
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<tbody>
<tr>
<td>S wave in subendocardium at the base (cm/s)</td>
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<tr>
<td>PIMO group**</td>
<td>5.3 ± 0.7</td>
<td>6.5 ± 1.4</td>
<td>7.8 ± 2.5</td>
<td>8.4 ± 1.5</td>
<td>9.4 ± 1.8</td>
<td>8.9 ± 0.6</td>
<td>7.8 ± 1.8</td>
<td>8.4 ± 1.2</td>
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<tr>
<td>BNZ group</td>
<td>6.6 ± 1.7</td>
<td>7.7 ± 1.6</td>
<td>6.0 ± 1.3</td>
<td>6.5 ± 1.8</td>
<td>7.0 ± 1.1</td>
<td>6.6 ± 1.3</td>
<td>6.8 ± 1.6</td>
<td>6.9 ± 1.4</td>
</tr>
</tbody>
</table>

S, peak velocity of the left ventricular free wall during systole.

*P < .05 versus BNZ group; **P < .001 versus BNZ group; ***P < .0001 versus BNZ group.

astolic diameter (Fig 2) associated with concomitant increases in systolic interventricular (P < .001) and left ventricular wall (P < .05) thicknesses (Fig 3). No change in LA/Ao ratio was observed. The systolic TDI variables also were significantly increased in the PIMO group, as compared to baseline, beginning at day 15 for the radial LVFW motion and day 49 for the longitudinal LVFW motion (Table 3).

An increase in the maximal anterior mitral valve leaflet thickness was observed in the PIMO group from day 414 to day 512 (7.5 ± 0.6 mm at day 512 versus 6.2 ± 0.7 mm at day 0; P < .05) but not in the BNZ group (6.1 ± 1.1 mm at day 512 versus 5.9 ± 0.9 mm at day 0). Moreover, at day 414 and day 512, respectively, 1 and 3 dogs in the PIMO group showed abnormal proliferative lesions on the atrial faces of the mitral leaflets. Such lesions were not observed in any of the dogs of the BNZ group (Fig 3). Finally, 3 dogs of the PIMO group had markedly echoic and irregular chordae tendineae, with an unusual thickening and irregular aspect mostly observed at their insertion on the leaflets (Fig 3).

Treatment Effects on Mitral Regurgitation, Aortic Peak Velocity, and Stroke Volume

All Doppler variables quantifying mitral regurgitation and aortic ejection flow remained unchanged throughout the study period in the BNZ group (Fig 4). Similarly, no significant increase in the aortic stroke volume or peak velocity was observed in the PIMO group (21.6 ± 3.0 mL at day 512 versus 19.0 ± 2.2 mL at day 0, and 1.5 ± 0.2 m/s at day 512 versus 1.4 ± 0.3 m/s at day 0, respectively). Conversely, treatment with PIMO was associated with a significant worsening of the mitral regurgitant jet as assessed by the ARJ/LAA ratio (P < .001), peak velocity (P < .001), and velocity time integral (P = .001) of the jet from day 15 to day 512 (Figs 3, 4). Moreover, as shown in Figure 5, the regurgitant jet duration increased during the study period in the PIMO group but not in the BNZ group.

Histopathologic Examination

As expected, MVD characterized by irregular, thickened, and sometimes opaque leaflets, mostly of the free

Fig 3. Two-dimensional echocardiography and Doppler examination from a representative dog from the benazepril (BNZ) group (A–C) and 1 from the pimobendan (PIMO) group (D–G) at the end of the study period. Note the severe reduction in the left ventricular cavity in the PIMO-treated dog (D) as compared with the BNZ-treated dog (A) in part because of the systolic hypertrophy of the papillary muscles (p). Note also, in the PIMO-treated dog, the hyperechoic endocardial surfaces of the left papillary muscles (D) and the increased thickness of the chordae tendineae and mitral leaflets with abnormal proliferative lesions (arrow) on the atrial faces (E) as compared with the BNZ-treated dog (B) on the right parasternal 4-chamber view. In the BNZ-treated dog, only a small early systolic mitral valve regurgitation without aliasing (arrow) was observed on the left parasternal 4-chamber view (C), whereas in the PIMO-treated dog, the regurgitant jet lasted the whole systole and was characterized by both a high maximal area of the regurgitant jet signal/left atrium area ratio (>70%, F) and a high maximal velocity (G). LA: left atrium; LV: left ventricle.
borders, was confirmed in all dogs of the study. However, the severity of the valvular lesions was higher in the PIMO group when compared to the BNZ group. The results of pathologic findings obtained by the board-certified pathologist and confirmed in a blinded fashion by the second pathologist are presented in Table 4. An increase in mitral valve glycosaminoglycans (GAG), characterized by multifocal accumulation of GAG in the valvular spongiosa, was noted in all animals. However, the severity grading of this change was higher in animals of the PIMO group when compared to the BNZ groups. Moreover, these GAG accumulations dissected the lamina fibrosa and extended to the valvular chordae tendineae in 3 animals of the PIMO group, but in no animal of the BNZ group (Fig 6A,B). Acute focal hemorrhages, characterized by the presence of extravasated red blood cells in the connective tissue just beneath the endocardium of the mitral valve leaflets, were noted in the mitral valve of 2 dogs treated with PIMO, but in no dog of the BNZ group. Hemosiderosis, corresponding to the presence of hemosiderin-laden macrophages (Berliner Blau staining positive) in the spongiosa of the mitral valve, was described with increased incidence and severity in the PIMO group when compared to the BNZ group (Fig 6C,D). Reactive valvular endothelium and chronic interstitial inflammation were described in the mitral valves of dogs from both groups. However, the incidence and severity of these findings were increased in the PIMO group when compared to the BNZ group. Three dogs from the PIMO group, but none from the BNZ group, demonstrated an endothelial irregular surface characterized by papillary projections bulging toward the left atrium (Fig 6E,F). Finally, dogs treated with PIMO showed moderate (n = 5) or slight (n = 1) endocardial sclerosis of the left papillary muscles mainly at the insertion of the chordae tendineae with local myocardial infiltration. These lesions were less severe and less common in the BNZ group (Table 4).

Discussion

This study demonstrates that long-term administration of PIMO in dogs with asymptomatic MVD is associated with an increase in systolic function and, concomitantly, a progressive worsening of MVD with development of specific mitral valve lesions. Conversely, long-term treatment with BNZ does not lead to adverse cardiac effects and is not associated with worsening of the valvular disease.

The present protocol has several major key features: the study was prospective, positive-controlled, blinded, and randomized with a parallel-group design involving dogs with spontaneous MVD and was conducted according to Good Clinical Practices (GCP) guidelines. Moreover, 1 trained observer, whose variability was known for all the assessed imaging variables, was involved for all imaging examinations.

Conventional echocardiography is a noninvasive imaging technique that enables the investigation of cardiac morphology, hemodynamics, and contractile function. Echocardiography is suitable for repeated measurements over time and thus enables easy noninvasive treatment follow-up. For the last 10 years, echocardiography has been increasingly used for toxicologic evaluation of drugs in laboratory animals. TDI was introduced as a new noninvasive and sensitive method for quantitatively analyzing segmental myocardial function. Myocardial velocities assessed by TDI reportedly are relatively independent of changes in ventricular loading conditions, and several TDI variables, particularly MVG, better reflect myocardial
function regardless of the presence of mitral regurgitation. For these reasons, TDI was chosen in the present study to complement conventional echocardiographic data and precisely assess the effect of PIMO on segmental myocardial systolic function. This approach is particularly important in MVD because mitral regurgitation is known to enhance %FS, thereby leading to an overestimation of inotropic function.

The first important finding of the present study is that PIMO treatment induces a marked and sustained increase in systolic myocardial function, with a sharp rise on day 15, as assessed by changes in M-mode parameters (eg, increased FS%, decreased left ventricular systolic diameter) and, most importantly, in systolic TDI variables (eg, higher systolic myocardial velocities and systolic radial MVG). This positive inotropic effect of PIMO is known to result from a dual mechanism of action: an increase in calcium sensitivity of cardiac myofilaments and inhibition of cAMP breakdown by PDE III. Surprisingly, the PIMO-induced hypercontractile state was not associated with a concomitant increase in arterial blood pressure and the maximal aortic flow velocity and stroke volume evaluated by Doppler measurements, which is thought to represent a logical hemodynamic correlate of an increase in left ventricular contractility. Conversely, the increased systolic myocardial function was associated with a major worsening of the mitral regurgitant jet characterized by a 3-fold increase in its extension in the left atrium and an increase in both its peak velocity and duration, thus explaining a 4-fold increase in the corresponding velocity time integral.

At day 0, none of the mitral regurgitant jets lasted the entire systole, and most of them were only observed in early systole, explaining the fact that most of the dogs showed low peak velocities of the mitral regurgitant jet, ie, <3 m/s. Peak velocity of the mitral regurgitant jet is directly related to the pressure difference (gradient, ΔP) across the mitral valve during systole, with ΔP = 4 × velocity² according to a modified Bernoulli equation. High-velocity mitral regurgitant jets (≥5 m/s) can only be observed when ΔP is maximal, ie, in mid systole when the systolic pressure in the left ventricle reaches at least 120–150 mmHg. From the onset of the ventricular mechanical systole to mid systole, the left ventricular pressure (and concomitantly ΔP across the mitral valve) gradually increases, leading to a progressive increase in the peak velocity of the mitral regurgitant jet. If the mitral regurgitation jet stops before mid systole, only low-peak regurgitant velocities are then recorded. This was the case for all dogs from the BNZ group at the end of the study period (day 512). Conversely, at day 512 all dogs from the PIMO group showed high peak velocities of the mitral regurgitant jet (≥5 m/s) because all regurgitant flows lasted at least until mid systole.

The PIMO-induced increase in mitral valve regurgitation observed in this study may partially be explained by ventricular hypercontractility and a secondary increase in systolic ventricular pressure, the upstream consequences of which were not limited by the possible arterial vasodilating properties of the drug. Peak mitral regurgitant velocities reached very high values in the PIMO-treated dogs (mean of 7.3 m/s on day 512). According to the modified Bernoulli equation, a mitral regurgitant jet velocity of 7.3 m/s corresponds to a pressure gradient across the mitral valve of 213 mmHg; this means that the mean left ventricular systolic pressure in the PIMO group was at least 213 mmHg, which is high compared to normal values (<160 mmHg). A similar marked increase in systolic left ventricular pressure (up to a >100% increase) was described after administration of inotropic drugs in the normal conscious dog without left ventricular pressure

Fig 5. The number of dogs in the benazepril (BNZ) (n = 6) and pimobendan (PIMO) (n = 6) groups according to duration of the regurgitation jet before initiation of therapy (day 0; A) and at day 512 (B). Dogs from the BNZ and the PIMO groups received 0.25 mg/kg PO q24h of benazepril and 0.25 mg/kg PO q12h of pimobendan, respectively. Early systolic jet, regurgitation jet ending before or at the end of the qRs complex; early and mid systolic jet, regurgitation jet ending before the middle of T wave; whole systolic jet, regurgitation jet lasting the whole systole (until the end of T wave).
Fig 6. Histopathology. (A, B) Representative photomicrographs of the mitral valves from dogs treated with 0.25 mg/kg q12h of pimobendan (PIMO) (A) or with 0.25 mg/kg q 24h of benazepril (BNZ) (B) for 512 days (Alcian Blue stain). Note the marked thickening of the mitral valve, particularly of the free border (fb) and the chordae tendinae (ct), by Alcian Blue positive glycosaminoglycans (GAG) in the PIMO-treated dog when compared to the BNZ-treated dog. (C, D) Representative photomicrographs of the mitral valves from dogs treated with 0.25 mg/kg q12h of pimobendan (PIMO) (C) or with 0.25 mg/kg q24h of benazepril (BNZ) (D) for 512 days (Prussian Blue stain). Note
overload, and bolus injections of PIMO have been shown experimentally to dramatically increase left ventricular pressure derivative (dP/dt) by up to 70%.

Such a high systolic ventricular pressure should have increased forward flow, aortic stroke volume, and arterial blood pressure. Surprisingly, systolic arterial blood pressure (indirectly assessed by the oscillometric method at the brachial artery) did not increase in the PIMO group. Similarly, Schneider et al observed jet lesions on the mitral valves without any increase in arterial blood pressure; indeed, in their study arterial blood pressure actually decreased. Several hypotheses may be proposed for these findings: (1) a dynamic left ventricular outflow tract obstruction that could not be detected using pulsed-wave Doppler mode because the sample gate was placed just at the level of the aortic valve (and not within the left ventricular outflow tract), (2) a decrease in left atrial pressure contributing to an increase in mitral regurgitant jet velocity (because PIMO decreases cardiac preload as a result of a venodilatation effect); (3) an underestimation of systolic blood pressure by the oscillometric method (although less probable according to published data on PIMO); and lastly (4) peripheral arterial vasodilatory properties of PIMO. Only direct (ie, invasive) blood pressure measurements could provide accurate information on the complex hemodynamic effects of PIMO on vascular beds and confirm or refute these hypotheses. A hypercontractility state is probably not the sole explanation of the increased mitral insufficiency with PIMO. The mitral valve apparatus is a complex structure, the proper function of which is dependent on the integrated performance of several elements: leaflets, annulus, chordae tendinae, and papillary muscles. One important finding of this study is the increased severity of the MVD lesions in the PIMO group associated with development of specific valvular lesions, thus complicating and worsening the initial disease. Such lesions (eg, acute hemorrhages, endocardial papilliform hyperplasia on the dorsal surfaces of the leaflets, infiltration of the chordae tendinae by GAG) were not detected in any dog of the BNZ group. These results corroborate the data obtained from 2 published toxicologic studies performed in dogs after single or repeated doses of PIMO. In the study of Schneider et al, proliferative jet lesions of the mitral valve and the left ventricular outflow tract endocardium were observed in 1 of 6, 2 of 6, and 6 of 8 healthy Beagle dogs after, respectively, 0.5, 2, and 8 mg/kg of PIMO injected IV once daily for 4 consecutive weeks. Hemorrhages of these cardiac structures also were found. According to the authors, all of these cardiac alterations were mainly a consequence of increased inotropic activity with secondary mechanical traumatic changes induced by vascular actions during systole. In other words, the cardiotoxicity of PIMO in dogs results mostly from the exaggerated pharmacodynamic effect rather than from the chemical nature of the compound per se. Similar lesions secondary to exaggerated pharmacodynamic activity also have been demonstrated in the dog with inotropic drugs other than PIMO. Results of all of these toxicologic studies with positive inotropic agents in dogs strongly support the hypothesis of a species-specific cardiotoxicity, with extremely high sensitivity of the dog (compared with humans, baboons, and rats) to the exaggerated pharmacodynamic effects and prolonged homeostatic disturbances induced by these drugs.

As previously described, the current study confirms that BNZ is well tolerated during long-term therapy in dogs with mild MVD and that no specific precautions appear to be necessary regarding plasma creatinine and urea concentrations or GFR. At the end of the study, GFR was determined to assess potential adverse effects of each tested treatment on renal function. GFR was significantly higher in the BNZ group as compared with the PIMO group. One limitation of the present study, however, is the absence of baseline data for GFR. The cause-effect relationship between the difference in GFR results and treatments cannot therefore be confirmed. Nevertheless, as previously reported with BNZ and other ACEI, long-term treatment with ACEI in dogs with MVD does not appear to affect renal function.

One limitation of the present study is that (for obvious reasons) no prestudy histologic examination of the mitral valve leaflets was available, and it was therefore impossible to know if treatment groups had similar histologic MVD-associated lesions at the beginning of the study period. However, it may be indirectly concluded that such was the case because the 2 groups showed similar echo-Doppler mitral regurgitant jet signals (eg, duration, maximum area, velocity time integral, and peak velocity) and similar thickness of the mitral leaflets. Furthermore, dogs were allocated to groups randomly; it is very unlikely that by chance alone the total lesion grade (assessed by blinded pathologists) was moderate to severe in half of the PIMO dogs, whereas it was minimal to slight in 100% of BNZ dogs or that none of the BNZ dogs showed accumulation of GAG in the chordae tendinae, whereas accumulation was moderate to severe in 50% of the PIMO dogs (Table 4). Last and more importantly, some valvular histopathologic lesions were observed in the PIMO group and not the presence of numerous hemosiderin-positive macrophages (arrows) in the zona auricularis and spongiosa of the mitral valve of the PIMO-treated dog. In comparison, no hemosiderophages are present in the BNZ-treated dog. (E, F) Representative photomicrographs of the mitral valves from dogs treated with 0.25 mg/kg q12h of PIMO (E) or with 0.25 mg/kg q24h of BNZ (F) for 512 days (hematoxylin and eosin stain). Note the reactive endothelium with papillary projections (arrows) on the atrial surface of the mitral valve of the PIMO-treated dog and, in comparison, the normal, smooth, endothelial surface in the BNZ-treated dog. za, zona auricularis; zs, zona spongiosa, if, lamina fibrosa.
in the BNZ group (eg, papillary projections bulging toward the left atrium). Such lesions cannot be explained by spontaneous valvular disease and have already been described in toxicologic studies performed in dogs after single or repeated doses of PIMO.\textsuperscript{29} Another limitation of this study is that no placebo group was used. This study design was chosen to compare the cardiac pharmacodynamics of PIMO and BNZ; therefore, only 2 groups of dogs were involved. Adding a placebo group would have provided more detailed information about the specific effects of the 2 drugs on disease progression. For example, during the 512 days of treatment there was no progression of murmur grades and no worsening of mitral regurgitation jet (eg, duration, peak velocity, velocity time integral, and extension within the LA) in the BNZ treated dogs; this observation may represent natural disease progression, but it also may reflect a beneficial BNZ effect that delayed worsening of the valvular lesions. Without any placebo group, no definitive conclusion on this particular point can be drawn, and additional studies are needed.

In conclusion, based on the present pathologic and Doppler data, the results of this study demonstrate that PIMO increases mitral valve regurgitation and induces specific mitral valve lesions. These results should encourage veterinarians to regularly and cautiously examine dogs affected by MVD and treated chronically with PIMO to check for any worsening of valvular lesions and regurgitation. Additional studies are now required to investigate the potential cardiotoxic adverse effects of PIMO on symptomatic canine MVD, particularly at early stages of congestive heart failure.

Table 4. Summary of histopathologic findings in dogs treated with 0.25 mg/kg q1h of pimobendan (PIMO group, n = 6) or with 0.25 mg/kg q24h of benazepril (BNZ group, n = 6) for 512 days.

<table>
<thead>
<tr>
<th>Lesions</th>
<th>No. of Dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td>MVD total lesion grade</td>
<td></td>
</tr>
<tr>
<td>PIMO group</td>
<td>0 0 3 2 1</td>
</tr>
<tr>
<td>BNZ group</td>
<td>0 2 4 0 0</td>
</tr>
<tr>
<td>Accumulation of glucosaminoglycans in</td>
<td></td>
</tr>
<tr>
<td>the chordae tendineae</td>
<td></td>
</tr>
<tr>
<td>PIMO group</td>
<td>3 0 0 2 1</td>
</tr>
<tr>
<td>BNZ group</td>
<td>6 0 0 0 0</td>
</tr>
<tr>
<td>Hemosiderosis (mitral leaflets)</td>
<td></td>
</tr>
<tr>
<td>PIMO group</td>
<td>0 2 2 2 0</td>
</tr>
<tr>
<td>BNZ group</td>
<td>3 3 0 0 0</td>
</tr>
<tr>
<td>Acute hemorrhage (mitral leaflets)</td>
<td></td>
</tr>
<tr>
<td>PIMO group</td>
<td>4 2 0 0 0</td>
</tr>
<tr>
<td>BNZ group</td>
<td>6 0 0 0 0</td>
</tr>
<tr>
<td>Reactive vascular endothelium (mitral leaflets)</td>
<td></td>
</tr>
<tr>
<td>PIMO group</td>
<td>2 2 2 0 0</td>
</tr>
<tr>
<td>BNZ group</td>
<td>3 3 0 0 0</td>
</tr>
<tr>
<td>Endothelial papillary projections (dorsal surface of the mitral leaflets)</td>
<td></td>
</tr>
<tr>
<td>PIMO group</td>
<td>3 1 2 0 0</td>
</tr>
<tr>
<td>BNZ group</td>
<td>6 0 0 0 0</td>
</tr>
<tr>
<td>Chronic interstitial inflammation (mitral leaflets)</td>
<td></td>
</tr>
<tr>
<td>PIMO group</td>
<td>0 4 2 0 0</td>
</tr>
<tr>
<td>BNZ group</td>
<td>3 3 0 0 0</td>
</tr>
<tr>
<td>Endocardial sclerosis (left papillary muscles)</td>
<td></td>
</tr>
<tr>
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<td>0 0 1 5 0</td>
</tr>
<tr>
<td>BNZ group</td>
<td>1 1 1 3 0</td>
</tr>
</tbody>
</table>

MVD, mitral valve disease.

Footnotes

\textsuperscript{a} Royal canin adult light, Royal Canin S.A, 30470 Aimargues, France
\textsuperscript{b} Fortekor, 5 mg benazepril HCl per tablet, Novartis Santé Animale, 68330 Huningue, France
\textsuperscript{c} Vetmedin, 2.5 mg and 5 mg pimobendan per tablet, Boehringer Ingelheim, 55216 Ingelheim, Germany
\textsuperscript{d} Dinamap, Critikon Inc, Tampa, FL
\textsuperscript{e} Cardiopac K130B, Camina SRL, Egna, Italy
\textsuperscript{f} Vivid 5, GE-Vingmed Ultrasound, Horten, Norway
\textsuperscript{g} Echo Pae 6.3 software for Vivid 5, GE-Vingmed Ultrasound, Horten, Norway
\textsuperscript{h} Systat version 10.0, SPSS Inc., Chicago, IL
Acknowledgment

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References


