Bepridil Inhibits Premature Ventricular Complexes Induced by Cardio-Sympathetic Nerve Stimulation in a Canine Experimental Model

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Summary

Sympathetic nerve activity has arrhythmogenic potential for ventricular arrhythmias associated with structural heart diseases. However, a sufficient amount of beta-blockers occasionally cannot be prescribed in some patients.

An experimental study was performed to clarify the therapeutic effects of bepridil, a multiple ionic current inhibitor that does not affect beta-adrenergic receptors, for premature beats occurring during enhanced sympathetic nerve activity. Cardio-sympathetic nerve activity was augmented via stellate-ganglion (SG) stimulation in a canine model (n = 8), and the arrhythmogenic potential and anti-arrhythmic effects of bepridil (2 and 4 mg/kg intravenously) were assessed. For safe use, vagal-stimulation-induced slow HR and programmed electrical stimulation were applied to evaluate possible pro-arrhythmic effects of the drug. Heart rate variability (HRV) indexes were used to estimate cardio-autonomic nerve activity.

Either side of the SG-stimulation increased BP and HR. Premature beats were induced in 10/16 SG-stimulations and it was more frequent in left (8/8) rather than right stimulation (2/8). Following 2 mg/kg drug administration, premature beats were still inducible in 8/16 stimulations (7/8 in left and 1/8 in right), but burden of the premature beats decreased from 87.1 ± 46.8 to 62.1 ± 42.6 beats. After 4 mg/kg administration, premature beats were inducible in one SG-stimulation. Proarrhythmic effects were not observed in all experiments. Steady-state HRV indexes and percent increases in SG-stimulation-induced BP-elevation and HR-acceleration were similar among the 3 periods (before, 2 and 4 mg/kg of the drug).

Bepridil may be an option for ventricular arrhythmias developed during enhanced cardio-sympathetic nerve activity with minimal effect on autonomic nerve responses.

Key words: Autonomic nerve activity, Ventricular arrhythmia

Implantable cardioverter defibrillators play a major role in the management of sustained ventricular arrhythmias. However, supplemental pharmacological therapies are usually needed to lower the risk of automatic shock delivery and improve prognosis and quality of life. It is well known that enhanced cardio-sympathetic activity facilitates the recurrence of sustained ventricular arrhythmias associated with structural heart diseases through altering arrhythmogenic substrate (conduction and repolarization) and inducing triggered premature beats. Therefore, beta-blockers and/or amiodarone have been used in suppressing these sustained ventricular arrhythmias. However, because of their potential side-effects and/or adverse effects (lung or thyroid toxicity, bradycardia, hypotension, etc.), these drugs cannot be prescribed in some patients. Like amiodarone, bepridil inhibits multiple myocardial ionic currents but without affecting beta-adrenergic receptors. Several clinical and experimental studies have reported the efficacy of bepridil for sustained ventricular arrhythmias, but these studies mostly focused on the effects on the myocardial substrate rather than the triggered premature beats.

To better clarify the roles of this drug as a supplemental, cardio-sympathetic activity was augmented through electrical stimulation of the stellate ganglion (SG) in a canine experimental model, and we studied whether the drug shows beneficial effects for triggered premature beats developed under the enhancement of sympathetic nerve activity. Inducibility of premature ventricular complex (PVC) and/or non-sustained ventricular tachycardia (NSVT, more than 3 consecutive PVC beats) and the therapeutic effects of two stepwise doses of bepridil (lower dose of 2.0 mg/kg and medium dose of 4.0 mg/kg intravenously) for PVC/NSVT were investigated using a systematic study protocol.
Methods

Surgical preparation: This study, approved by the animal studies subcommittee of our institutional review board, was in compliance with the guidelines of the United States National Institutes of Health for the Care and Use of Laboratory Animals. The experiments were performed in 8 intubated and artificially ventilated beagles, weighing between 11.0 and 14.0 kg, anesthetized with a 17.5-mg/kg intravenous bolus, followed by a maintenance dose of 3.0-5.0 mg/kg/hour, of sodium thiamylal. Pentazocine, 2 mg/kg, was also administered intravenously and the Bispectral index was maintained between 40 and 60. Catheters were inserted into the femoral vein for administration of fluids and drugs, and into the femoral artery to monitor arterial blood pressure (BP). A surface electrocardiogram (Lead II) was monitored and the core body temperature was kept at 37°C with a thermostatically controlled blanket. To stimulate the cardiac sympathetic nerve, left- and right-sided stellate ganglions (L-SG and R-SG) were exposed via a midline sternotomy, and a pair of polyimide-coated silver wires were inserted into each SG.15) Another pair of stimulation wires were inserted into the right cervical vagosympathetic trunk to obtain the slow heart rate (HR). For applying programmed electrical stimulation (PES) and back-up pacing during vagal-stimulation-induced slow HR, bipolar electrodes were placed in the right ventricular endocardial site, and the heart was paced at twice the end-diastolic threshold by a cardiac stimulator. After these preparations, 1000 units of heparin sodium was administered intravenously followed by 200 units every hour. Upon completion of the experimental protocol, the animals were sacrificed by electrical induction of ventricular fibrillation under deep general anesthesia.

Measurements of heart rate variability (HRV): The body surface electrocardiogram was digitized at a sampling rate of 1,000 Hz by an ADX-002 analog-to-digital converter (ADTEC Inc., Tokyo) and stored in a personal computer. The cardiac cycle length was measured using GM-View II R-wave detection software (Signalysis Ltd., Saitama, Japan). The MemCalc method was used to calculate the high-frequency (HF; 0.156-0.406 Hz) and low-frequency (LF; 0.047-0.156 Hz) components of HRV, using HPS-RRA, version 02-01, software (Fukuda Denshi Co., Tokyo). In this study, the LF and HF components are expressed as amplitude [LF-amp = (2 • LF component)1/2] and HF-amp = (2 • HF component)1/2] and the LF/HF ratio was calculated.

Study protocol and data collection: Data collection was performed 3 times during the experiments; before administration of bepridil, 5 minutes after completion of initial drug administration (lower dose; 2.0 mg/kg intravenously), and 5 minutes after completion of the additional 2.0 mg/kg of drug administration (medium dose; 4.0 mg/kg intravenously in total). This is because previous experimental studies usually tested 3-5 mg/kg of bepridil intravenously.16-18) Bepridil, which was dissolved in dimethyl sulfoxide and diluted into 10 mL of sterile saline, was then administered intravenously within 3 minutes.

The following parameters were assessed at each data collection; HR, BP, ECG parameters, indexes of HRV, effective refractory period (ERP) of the right ventricle, and inducibility of VA. Average values of PQ interval, QRS-duration, QT and T-peak-T-end (Tp-e)19) intervals, and corrected QT and Tp-e (QTc and Tcp-e) intervals on the surface ECG were calculated from 3 consecutive cycles. If PVC/NSVT occurred during the measuring period, each parameter was calculated after the PVC/NSVT disappeared. In order to confirm the stable drug effects, QT/QTc and Tp-e/Tcp-e intervals were measured both before and after attempting the provocation tests (described below) in each data collection time. Three methods were applied in order to assess the inducibility of ventricular arrhythmias. (1) Slow HR. HR was slowed to 40-60 bpm by use of electrical vagal stimulation (10 Hz, 5 ms, 3-5 mA) and maintained for 60 sec. This was because bepridil prolongs the QT interval, and marked QT interval prolongation under slow HR may induce polymorphic ventricular arrhythmias. (2) PES. PES with 1-2 extrastimuli and burst pacing up to 210 bpm were attempted from the right ventricle. (3) Sympathetic stimulation. The R-SG or L-SG was stimulated for 30 seconds by an electrical stimulator (10 Hz, 5 ms, 10 mA). R-SG stimulation was attempted first because PVC/NSVT were expected to be induced more frequently by L-SG stimulation, and approximately 10 minutes after the R-SG stimulation, once BP and HR had returned to their previous levels, L-SG stimulation was attempted. Changes in BP and HR due to SG-stimulation were assessed during this period.

Total numbers of extra-systolic-beats (EB-burden: total beats of PVC+NSVT) and maximum continuous beats of NSVT (max-NSVT) were calculated in each induction by high speed ECG recordings. The therapeutic effects of bepridil for these premature beats were assessed by use of the following two criteria. Complete suppression was defined as when the PVC and NSVT became non-inducible after drug administration. Partial suppression was indicated when EB-burden decreased more than 50% and max-NSVT was shortened more than 50% as compared to those before administration of the drug. All the protocols were accomplished within 150 minutes after completion of the surgical preparation and approximately 90 minutes after starting the initial drug administration.

Statistical analysis: The measurements are presented as the mean ± standard deviation. Paired t-tests were used to compare BP and HR before and after SG-stimulation in each data collection. QT/QTc and Tp-e/Tcp-e intervals before and after the provocation protocol in each data collection time were also compared by this test. Changes in EB-burden and max-NSVT before and after bepridil (2 mg/kg) were assessed using Fisher’s exact test. Steady-state values of BP, HR, HRV indexes, and ECG parameters among the 3 periods of the data collection were compared by analysis of variance (ANOVA) and the Scheffe multiple-range post hoc test, where appropriate, using SPSS software version 14 (SPSS Institute Inc., Chicago, IL, USA). The percent increases of the SG-stimulation-induced rises in HR and BP among the 3 periods of data collection were also assessed by the same test. Cochran’s Q-tests were used for comparison of the PVC/NSVT inducibility among the 3 periods of data collection. A P value < 0.05 was considered statistically significant.
Results
BP, HR, ECG parameters, ERP and HRV indexes during the steady-state: Steady-state systolic/diastolic BP and HR before administration of the drug were 134 ± 13/92 ± 15 mmHg and 114 ± 18 bpm, respectively. Lower and medium doses of the drug decreased the steady-state BP (132 ± 14/85 ± 14 mmHg at lower dose and 125 ± 19/71 ± 20 at medium dose, \( P < 0.001 \) by ANOVA) and slowed the HR (93 ± 16 bpm at lower dose and 84 ± 9 bpm at medium dose, \( P < 0.01 \) by ANOVA) at the beginning of provocation tests in each data collection time. The QT/QTc intervals and Tp-e/Tcp-e intervals were not different before and after performing the provocation tests in each data collection time (Table I). Bepridil prolonged the PQ interval (from 98 ± 15 to 106 ± 13 ms at lower and to 112 ± 14 ms at medium dose, \( P < 0.001 \) by ANOVA) (Table I). Comparisons before and after provocation protocol
QT, ms 291 ± 56 341 ± 84 389 ± 80 < 0.001 < 0.001 < 0.001 < 0.001
QTc, ms 391 ± 70 424 ± 98 477 ± 108 < 0.001 < 0.001 < 0.001 < 0.001
Tp-e, ms 58 ± 19 80 ± 21 113 ± 24 < 0.001 < 0.001 < 0.001 < 0.001
Tcp-e, ms 105 ± 25 138 ± 23 180 ± 33 < 0.001 < 0.001 < 0.001 < 0.001
ERP, ms 188 ± 8 214 ± 15 226 ± 13 < 0.001 < 0.001 < 0.001 < 0.001
LF/HF 0.61 ± 0.53 0.54 ± 0.40 0.40 ± 0.34 0.061 0.665 0.054 0.222
ErP, ms 188 ± 8 214 ± 15 226 ± 13 < 0.001 < 0.001 < 0.001 < 0.001
HR, bpm 114 ± 18 93 ± 16 84 ± 9 < 0.001 < 0.001 < 0.001 < 0.001

Comparisons before and after provocation protocol
QT/QTc 0.495/0.405 0.540/0.400 0.400/0.340 0.061 0.665 0.054 0.222
TP-e/TCP-e 1.000/0.741 0.711/0.447 0.523/0.879

Results
BP, HR, ECG parameters, ERP and HRV indexes during the steady-state: Steady-state systolic/diastolic BP and HR before administration of the drug were 134 ± 13/92 ± 15 mmHg and 114 ± 18 bpm, respectively. Lower and medium doses of the drug decreased the steady-state BP (132 ± 14/85 ± 14 mmHg at lower dose and 125 ± 19/71 ± 20 at medium dose, \( P < 0.001 \) by ANOVA) and slowed the HR (93 ± 16 bpm at lower dose and 84 ± 9 bpm at medium dose, \( P < 0.01 \) by ANOVA) at the beginning of provocation tests in each data collection time. The QT/QTc intervals and Tp-e/Tcp-e intervals were not different before and after performing the provocation tests in each data collection time (Table I). Bepridil prolonged the PQ interval (from 98 ± 15 to 106 ± 13 ms at lower and to 112 ± 14 ms at medium dose, \( P < 0.001 \) by ANOVA) and did not show statistical differences in QRS duration (from 98 ± 3 to 99 ± 4 ms at lower dose and to 99 ± 3 ms at medium dose, \( P = 0.073 \) by ANOVA) at the beginning of provocation tests in each data collection time. The QT/QTc intervals and Tp-e/Tcp-e intervals were prolonged in a dose-dependent manner (QT/QTc intervals: from baseline at 286 ± 57/391 ± 70 to 339 ± 88/424 ± 98 ms at lower dose and to 402 ± 92/± 108 ms at medium dose, \( P < 0.001 \) by ANOVA). After administration of the drug, PVC/NSVT were still inducible in 1 of the 8 L-SG stimulations (100%) (Table II) (Figures 2-4). The PVC/NSVT inducibility by L-SG was greater than that of PVC/NSVT inducibility by R-SG (\( P = 0.007 \)). After administration of the lower dose of bepridil, PVC/NSVT were still inducible in one of the 8 R-SG stimulations (12.5%) and in 7 of the 8L-SG stimulations (87.5%). However, in these 8 stimulations (one in R-SG and 7 in L-SG), EB-burden and max-NSVT decreased from 87.1 ± 46.8 to 62.1 ± 42.6 (\( P = 0.036 \)) and from 69.6 ± 59.1 to 32.5 ± 33.6 (\( P = 0.036 \)).

### Table I. Steady-State Parameters before and after Bepridil

<table>
<thead>
<tr>
<th>Parameters before and after Bepridil</th>
<th>Before (B)</th>
<th>Lower dose (L)</th>
<th>Medium dose (M)</th>
<th>Comparisons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP, mmHg</td>
<td>134 ± 13</td>
<td>132 ± 14</td>
<td>125 ± 19</td>
<td>ANOVA B versus L</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>92 ± 15</td>
<td>85 ± 14</td>
<td>71 ± 20</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>114 ± 18</td>
<td>93 ± 16</td>
<td>84 ± 9</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>PQ interval, ms</td>
<td>98 ± 15</td>
<td>106 ± 13</td>
<td>112 ± 14</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>QRS duration, ms</td>
<td>98 ± 3</td>
<td>99 ± 4</td>
<td>99 ± 3</td>
<td>0.073</td>
</tr>
<tr>
<td>QT, ms</td>
<td>286 ± 57</td>
<td>339 ± 88</td>
<td>402 ± 92</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>QTc, ms</td>
<td>391 ± 70</td>
<td>424 ± 98</td>
<td>477 ± 108</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Tp-e, ms</td>
<td>58 ± 19</td>
<td>80 ± 21</td>
<td>113 ± 24</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Tcp-e, ms</td>
<td>105 ± 25</td>
<td>138 ± 23</td>
<td>180 ± 33</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>ERP, ms</td>
<td>188 ± 8</td>
<td>214 ± 15</td>
<td>226 ± 13</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>LF/HF 0.61 ± 0.53</td>
<td>0.54 ± 0.40</td>
<td>0.40 ± 0.34</td>
<td>0.061</td>
<td>0.665</td>
</tr>
<tr>
<td>HF-amplitude, ms</td>
<td>2.17 ± 0.37</td>
<td>2.77 ± 1.04</td>
<td>3.03 ± 0.53</td>
<td>0.193</td>
</tr>
</tbody>
</table>
| Comparisons before and after provocation protocol
| QT/QTc                             | 0.495/0.405     | 0.673/0.766     | 0.099/0.087     |
| Tp-e/Tcp-e                         | 1.000/0.741     | 0.711/0.447     | 0.525/0.879     |

Values are means ± SD. HR indicates heart rate; BP, blood pressure; ERP, effective refractory period; LF, low-frequency components; HF, high-frequency components; TP-e, Tpeak-Tend interval; and Tcp-e, corrected Tpeak-Tend interval.

Before administration of the drug, PVC/NSVT were induced in 2 of the 8 R-SG stimulations (25%) and in all 8 L-SG stimulations (100%) (Table II) (Figures 2-4). The PVC/NSVT inducibility by L-SG was greater than that of PVC/NSVT by R-SG (\( P = 0.007 \)). After administration of the lower dose of bepridil, PVC/NSVT were still inducible in one of the 8 R-SG stimulations (12.5%) and in 7 of the 8L-SG stimulations (87.5%). However, in these 8 stimulations (one in R-SG and 7 in L-SG), EB-burden and max-NSVT decreased from 87.1 ± 46.8 to 62.1 ± 42.6 (\( P = 0.036 \)) and from 69.6 ± 59.1 to 32.5 ± 33.6 (\( P = 0.036 \)).
Figure 1. Slow heart rate (HR) application. HR was slowed to 40-60 bpm using cervical vagosympathetic trunk electrical stimulation. No premature ventricular complex (PVC) occurred during the slow HR before (A) and after administration of bepiridil (lower dose in B and medium dose in C).

Table II. Changes in Stellate Ganglion Stimulation-Induced BP, HR and PVC/NSVT

<table>
<thead>
<tr>
<th></th>
<th>Before (B)</th>
<th>Lower dose (L)</th>
<th>Medium dose (M)</th>
<th>Comparisons of increase values among the three conditions</th>
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<tbody>
<tr>
<td><strong>Right</strong></td>
<td></td>
<td></td>
<td></td>
<td>ANOVA (Cochran) B versus L B versus M L versus M</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>132 ± 13 to 162 ± 10</td>
<td>133 ± 15 to 160 ± 17</td>
<td>127 ± 17 to 148 ± 14</td>
<td>0.476 0.740 0.449 0.875</td>
</tr>
<tr>
<td>percent increase, %</td>
<td>+ 24 ± 12</td>
<td>+ 21 ± 14</td>
<td>+ 19 ± 20</td>
<td></td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>90 ± 15 to 112 ± 11</td>
<td>85 ± 15 to 102 ± 17</td>
<td>72 ± 20 to 82 ± 18</td>
<td>0.303 0.771 0.274 0.639</td>
</tr>
<tr>
<td>percent increase, %</td>
<td>+ 26 ± 15</td>
<td>+ 23 ± 18</td>
<td>+ 18 ± 17</td>
<td></td>
</tr>
<tr>
<td>HR, bpm</td>
<td>113 ± 19 to 170 ± 23</td>
<td>93 ± 15 to 139 ± 25</td>
<td>84 ± 10 to 118 ± 19</td>
<td>0.217 1.000 0.272 0.283</td>
</tr>
<tr>
<td>percent increase, %</td>
<td>+ 52 ± 19</td>
<td>+ 52 ± 27</td>
<td>+ 41 ± 27</td>
<td>(0.223)</td>
</tr>
<tr>
<td>PVC/NSVT (%)</td>
<td>2 (25)</td>
<td>1 (12.5)</td>
<td>0 (0)</td>
<td></td>
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<tr>
<td><strong>Left</strong></td>
<td></td>
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</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>135 ± 14 to 189 ± 17</td>
<td>131 ± 15 to 194 ± 23</td>
<td>124 ± 22 to 182 ± 20</td>
<td>0.312 0.408 0.351 0.993</td>
</tr>
<tr>
<td>percent increase, %</td>
<td>+ 41 ± 18</td>
<td>+ 50 ± 27</td>
<td>+ 51 ± 35</td>
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<tr>
<td>Diastolic BP, mmHg</td>
<td>93 ± 16 to 108 ± 15</td>
<td>85 ± 14 to 101 ± 16</td>
<td>71 ± 22 to 83 ± 27</td>
<td>0.546 0.522 0.912 0.767</td>
</tr>
<tr>
<td>percent increase, %</td>
<td>+ 17 ± 9</td>
<td>+ 19 ± 10</td>
<td>+ 18 ± 13</td>
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</tr>
<tr>
<td>HR, bpm</td>
<td>115 ± 19 to 137 ± 21</td>
<td>94 ± 18 to 121 ± 26</td>
<td>84 ± 8 to 108 ± 24</td>
<td>0.067 0.071 0.159 0.890</td>
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<tr>
<td>percent increase, %</td>
<td>+ 20 ± 15</td>
<td>+ 30 ± 23</td>
<td>+ 28 ± 24</td>
<td>(0.002)</td>
</tr>
<tr>
<td>PVC/NSVT (%)</td>
<td>8 (100)</td>
<td>7 (87.5)</td>
<td>1 (12.5)</td>
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</table>

Comparisons of percent increases right and left

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<table>
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<tbody>
<tr>
<td>Systolic BP</td>
<td>0.008</td>
<td>0.013</td>
<td>0.008</td>
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<tr>
<td>Diastolic BP</td>
<td>0.077</td>
<td>0.638</td>
<td>0.983</td>
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</tr>
<tr>
<td>HR</td>
<td>0.009</td>
<td>0.196</td>
<td>0.424</td>
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Values are means ± SD. BP indicates blood pressure; HR, heart rate; PVC, premature ventricular contraction; and NSVT, non-sustained ventricular tachycardia.
Figure 2. Right-side stellate-ganglion (SG) stimulation. Before drug, SG-stimulation on the right-side elevated BP and accelerated HR (A), and similar BP and HR responses were reproduced after administration of lower and medium doses of bepridil (B and C).

0.039), respectively (Figure 4). After administration of the medium dose of the drug, PVC/NSVT were non-inducible in all R-SG stimulations and only inducible in one L-SG stimulation. In this L-SG stimulation, EB-burden and max-NSVT decreased from 176 and 162 (before drug) to 3 and 1 (after medium dose), respectively. Inducibility of PVC/NSVT decreased following drug administration in the L-SG stimulation (P = 0.002) but not in the R-SG stimulation (P = 0.223) (Table II).

There was no experiment in which PVC/NSVT became inducible only after administration of the drug. Sustained ventricular arrhythmias were not induced by any SG-stimulation in any experiment. After cessation of the SG-stimulations, the induced PVC/NSVT gradually disappeared, and together with BP and HR returned to the baseline values. On the whole, the lower dose of bepridil showed some therapeutic effects for PVC/NSVT in 5 of the 10 stimulations (50%) (complete suppression in 2 and partial suppression in the other 3 stimulations). On the other hand, the medium dose of bepridil demonstrated therapeutic effects for PVC/NSVT in all 10 of the 10 stimulations (100%) (complete suppression in 9 and partial suppression in the remaining one stimulation) (Table II).

SG-induced BP augmentation and HR acceleration: Before administration of bepridil, R-SG stimulation increased BP from 132 ± 13/90 ± 15 to 162 ± 10/112 ± 11 mmHg (P < 0.001), and accelerated HR from 113 ± 19 to 170 ± 23 bpm (P < 0.001). L-SG stimulation also elevated BP from 135 ± 14/93 ± 16 to 189 ± 17/108 ± 15 mmHg (P < 0.001) and accelerated HR from 115 ± 19 to 137 ± 21 bpm (P = 0.005). The percent increases in systolic BP were greater in L-SG stimulation (P = 0.008), whereas the percent increases in HR acceleration were more obvious in R-SG stimulation (P = 0.009).

The percent increases in BP (systolic and diastolic) and HR were not statistically different among the 3 data collection times in each side of the SG stimulation. The percent increases in BP and HR in the R-SG stimulation were +24 ± 12/+26 ± 15% and +52 ± 19% before, +21 ± 14/+23 ± 18% and +52 ± 27% after the lower dose, and +19 ± 20/+18 ± 17% and +41 ± 27% after medium dose administration, respectively (P = 0.476/0.303 for BP and P = 0.217 for HR by ANOVA). The percent increases in BP and HR in the L-SG stimulation were +41 ± 18/+17 ± 9% and +20 ± 15% before, +50 ± 27/+19 ± 10% and +30 ± 23% after the lower dose, and +51 ± 35/+18 ± 13% and +28 ± 24% after medium dose administration, respectively (P = 0.312/0.546 for BP and P = 0.067 for HR by ANOVA) (Table II).

Discussion

The main findings of this study were 1) L-SG rather than R-SG stimulation induced PVC/NSVT more frequently, and intravenous administration of bepridil showed inhibitory effects for these arrhythmias in a dose dependent manner, 2) neither dosage of the drug showed proar-
Figure 3. Left-side stellate-ganglion (SG) stimulation. Before drug administration, SG-stimulation on the left-side elevated BP, accelerated HR and induced premature ventricular complex (PVC) and non-sustained ventricular tachycardia (NSVT) (A). These PVC/NSVT were partially inhibited by the lower dose (B) and completely suppressed after medium dose administration of the drug (C). Similar BP and HR responses were reproduced after lower and medium doses of bepridil (B and C). This experiment was different from that shown in Figure 2.

rhythmic effects, and neither polymorphic ventricular arrhythmias associated with QT interval prolongation nor PES-induced sustained ventricular arrhythmias were observed in any of the experiments, and 3) intravenous bepridil administration decreased steady-state BP and HR without changing the values of HRV indexes, and the same magnitudes of the SG-stimulation-induced BP-augmentation and HR-acceleration as those observed before were reproduced after administration of the drug.

SG-stimulation and cardio-sympathetic nerve activation: R-SG or L-SG stimulation alone both accelerated HR, elevated BP and induced PVC/NSVT, but the inducibility of PVC/NSVT and magnitude of the BP rises were greater with L-SG stimulation, findings which are similar to previous studies.20) The left- and right-side specific differences can be explained by the distribution of cardio-sympathetic nerves within the heart, as the left-side nerves primarily run into the left atrium and ventricle whereas the right-side nerves are predominantly distributed through the right atrium (including sinus node) and ventricle.21)

Drug effects for SG-stimulation induced PVC/NSVT: The occurrence and disappearance of the PVC/NSVT were associated with the BP and HR alteration induced by SG-stimulation. Therefore, automaticity and/or triggered activity, stimulated by the cardio-sympathetic nerve activation, are the likely causes of the SG-stimulation-induced PVC/NSVT. Since bepridil does not have beta-blocking action, suppression of the SG-stimulation-induced PVC/NSVT by bepridil was probably caused by its inhibitory effects on calcium current. Bepridil is known to have multiple channel blocking action (including sodium and calcium currents, delayed rectifier potassium current, ultra-rapid potassium current, transient outward potassium current, inward rectifying potassium current, potassium-adenosine triphosphate current and hyperpolarization-activated non-selective cation current),8-10) and inhibition of calcium current by bepridil in this study is represented as the observed results of (1) prolonged steady-state HR and PQ interval and (2) lowered steady-state BP. Indeed, several studies reported that calcium channel blockers had suppressive effects on PVC during sympathetic nerve activation.22) Bepridil prolongs myocardial repolarization through the inhibition of several potassium currents, and this is considered the main antiarrhythmic action for reentrant arrhythmias during this drug therapy. It is possible that such repolarization effects of this drug were associated with the inhibition of the SG-stimulation induced PVC/NSVT. Since a likely mechanism of the PVC/NSVT was non-reentry rather than reentry,23) we think that the primary reason for this antiarrhythmic effect is due to calcium current inhibition. However, it seems more reasonable to consider that multiple ionic current inhibition by bepridil prevented the SG-stimulation induced PVC/NSVT in this experimental study.

On the other hand, the following two observations of this study were consistent with the previous result that
bepridil did not affect beta-adrenergic receptors; no difference in the (1) steady-state LF/HF and HF-amp values and (2) magnitude of BP-elevation and HR-acceleration by SG-stimulation, among the 3 periods of data collection (Table II). HRV has been used for the analysis of cardio autonomic nerve activity in various situations (including during treatment with antiarrhythmic drugs).24,25 Although to the best of our knowledge no results for HRV during bepridil treatment in clinical cases have been published, there seems to be no specific reason why HRV is inapplicable during bepridil treatment. Identifying detailed ionic mechanisms for suppression of SG-stimulation induced PVC/NSVT is important. However, this subject could not be directly addressed based on the results of this in vivo experimental study.

Before administration of the drug, SG-stimulation-induced PVC/NSVT gradually disappeared after cessation of the SG-stimulation without deteriorating into sustained ventricular arrhythmias. This was probably because we used healthy beagles without any structural heart diseases. However, it seems reasonable that these PVC/NSVT can trigger frequent sustained ventricular arrhythmias episodes in patients suffering from cardiac dysfunction due to structural heart diseases. Since PVC/NSVT induced by cardio-sympathetic nerve activation were successfully inhibited in this study, the medium rather than lower dose of bepridil may protect against electrical storms, which usually develop in a background of heightened sympathetic nervous activity.

**Safe usage of this drug:** Since bepridil prolonged the QT/QTc and Tp-e/Tcpe intervals (402 ± 92/477 ± 108 ms and 113 ± 24/180 ± 33 ms after administration of medium dose, respectively), polymorphic ventricular arrhythmias may develop as a proarrhythmic effect of this drug, especially during bradycardia conditions.26 The Tp-e/Tcpe interval has been reported to represent transmural dispersion of ventricular repolarization.27

For safe use of this drug, we slowed the HR by cervical vagosympathetic stimulation, and confirmed that no sustained ventricular arrhythmias were induced during bradycardia. PES also failed to induce any sustained ventricular arrhythmias after either dosage of the drug was administered. In the ventricle, the rapid component of the delayed rectifier potassium current (I_{kr}) is a major repolarizing current during bradycardia, whereas the slow component of the delayed rectifier potassium current (I_{ks}) becomes more important at faster heart rates. Therefore, the inhibition of only I_{kr} was associated with a lesser increase in repolarization at faster pacing rates and a greater increase at slower HR. On the other hand, as with amiodarone, the inhibition of both I_{kr} and I_{ks} concomitant with the suppression of calcium, sodium and several potassium currents by bepridil, may participate in the less arrhythmogenic potential during slower HR. In our previous study using the same model, bradycardia-dependent QT interval prolongation and repolarization dispersion increment were less apparent with bepridil as compared to a pure I_{kr} blocker. These results from our present and pre-
arious studies may suggest that bepridil, up to a medium dosage, could be safely used for the treatment of ventricular arrhythmias. While greater amounts, of course, have a potential risk of polymorphic ventricular arrhythmias, this drug was deemed to have been used in a safe therapeutic range in this experiment.

In addition, it is important to note that the beneficial results of bepridil in this experiment cannot be simply applied to clinical treatment, because our results were obtained from intravenous administration of the drug in a canine experimental model, and the methods are quite different from clinical usage of the drug.

**Clinical implications:** Beta-blockers and amiodarone are the first-line supplemental drugs in the prevention of ventricular arrhythmia recurrence in patients with structural heart diseases. The results of this experimental study suggest the possibility that bepridil can be used as an alternative in those patients in whom the first-line drugs are unable to be prescribed due to various reasons. But this possibility needs to be confirmed in future clinical studies.

**Study limitations:** This study has several limitations. First, the effects of bepridil were evaluated after short-term intravenous administration of the drug, and we did not measure the serum concentration. Although previous studies reported a reasonable serum concentration with a similar drug administration method as we used in this study, long-term administration of the drug may show different electrophysiological effects. However, since the QT/QTc and Tp-e/Tcp-e intervals before and after attempting the provocation tests were stable for each data collection time, it is reasonable to think that inhibition of the SG-simulation induced PVC/NSVT was due to the effects of bepridil. Second, we attempted either right- or left-side SG-stimulation sequentially, but simultaneous simulation from both sides of the SG seems to be more clinically relevant. However, cardio-sympathetic nerve activity was augmented by stimulation on either side of the SG. Third, it was performed in dogs with normal hearts, while the inducibility of ventricular arrhythmias and the effects of the drug might differ in diseased myocardium. Fourth, this study focused on the therapeutic effects for triggered PVC/NSVT induced by sympathetic stimulation. As a multi-channel blocker, bepridil would be expected to show some beneficial effects on the myocardial substrate. However, the drug’s effects on the myocardium had already been examined in several previous studies by ourselves and other researchers. Therefore, we focused on the triggered premature beats in this study.

**Conclusions**

Intravenous administration of bepridil has suppressive effects on sympathetic nerve activity-induced PVC/NSVT while it exhibits little effect on autonomic nerve activity.

**Disclosure**

Conflicts of interest: The authors have no potential conflict of interest to disclose.

References