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Effects of Rotigaptide, a Gap Junction Modifier, on Defibrillation Energy and Resuscitation From Cardiac Arrest in Rabbits

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The gap junction modifier Rotigaptide (ZP123), which promotes cellular coupling, was hypothesized to decrease defibrillation thresholds during prolonged ventricular fibrillation (VF). Thirty-two New Zealand white rabbits were randomized to receive saline (control, n = 16) or Rotigaptide (n = 16). Following 4 min of untreated VF, biphasic defibrillation shocks were applied through chest wall patches, starting either at 300 volts (V) (n = 16) or 500 V (n = 16), with 200 V increasing steps to 900 V in case of shock failure. Rotigaptide significantly decreased defibrillation voltage requirements (average cumulative voltage of all shocks: 1206 ± 709 V in control group vs. 844 ± 346 V in treated group, P = .002). Rotigaptide had no effect on heart rate, QRS duration, QT interval, ventricular effective refractory period, monophasic action potential duration or on connexin 43 density using immunofluorescence. Rotigaptide improves the ability to defibrillate after untreated VF.

Keywords: gap junctions; ventricular fibrillation; defibrillation; connexin 43

Ventricular fibrillation (VF) is the leading cause of sudden cardiac death in the industrialized world.1 Defibrillation threshold and the risk of re-fibrillation increases as the duration of VF increases.1,2 Higher energies required for defibrillation are associated with poor outcomes.2,3 These phenomena may be due to tissue hypoxia, myocardial ischemia, and acidosis that progressively worsens as VF continues.

- Pharmacologic therapy to prevent or treat VF (as an adjunct to defibrillation) using currently available anti-arrhythmic agents has been limited by lack of efficacy and pro-arrhythmic effects.4-6 No drug has been shown to improve survival to discharge following out-of-hospital VF.
- Gap junctions are low resistance connections between adjacent myocardial cells, consisting of proteins, most predominantly connexin 43 (Cx43), which mediate current transfer between cells and promote rapid conduction of depolarizing wave-fronts during normal cardiac electrical activation. Disruption of gap junction coupling during pathological conditions results in discontinuous or slowed conduction and may increase the risk for re-entrant arrhythmias.7
- Changes in metabolic factors during myocardial ischemia, and possibly during VF, induces intercellular gap junction uncoupling, and may contribute to conduction slowing and wavelet fragmentation during VF.7,8 If gap junction uncoupling could be prevented, the adverse electrophysiological consequences of prolonged VF may also be modified. Previous studies demonstrated that decreased conduction velocity after sodium channel blocker administration (eg, lidocaine) is consistently linked to increased defibrillation threshold in animals and...
Based on these findings, we hypothesized that agents that reduce the extent of conduction velocity slowing during VF may have the potential to reduce defibrillation threshold (DFT).

- **Rotigaptide (ZP123),** a new anti-arrhythmic peptide, was recently found to reduce gap junction closing during acidosis, and to prevent intercellular uncoupling, which causes conduction velocity slowing and heterogeneous repolarization, without changing membrane conductance.

- We therefore studied the effect of the gap junctional modifier rotigaptide on defibrillation thresholds during prolonged electrically induced VF in an in vivo rabbit model.

### Methods

Thirty-two healthy male rabbits (3.6-4.8 kg, male, New Zealand White) were used in this study. The protocol was approved by the Animal Care Committee of St. Michael's Hospital and conformed to the guiding principles of the Canadian Council on Animal Care. Rabbits were anesthetized with xylazine (4 mg/kg) and ketamine (20 mg/kg) injected intramuscularly and maintained with isoflurane (0.5% to 3%) inhalation. Tracheotomy was performed with a 3F sheath. The rabbits were ventilated by an Ohmeda® 7800 ventilator (Ohmeda, Madison, WI), with a tidal volume of 10 mL/kg and a rate between 20 and 25 breaths/minute. End-tidal carbon dioxide (EtCO2) was recorded continuously (LIFEPAK 12TM, Medtronic-Physio Control, Redmond, WA) and maintained at a level of 35 to 40 mmHg.

- The left carotid artery and right jugular vein were isolated by blunt dissection and cannulated. Blood pressure recordings and blood samples were obtained from the artery. Carotid arterial blood pressure was continuously measured with a fluid-filled transducer (Hewlett Packard, USA). A monophasic action potential (MAP) catheter (EP Technologies Inc., Sunnyvale, CA) was inserted through the right femoral vein into the right ventricle for the recording of MAPs and inducing VF. Stable endocardial contact was verified by MAP morphology. Signals were filtered at DC-500 Hz and stored for offline analysis. Surface electrocardiographs (ECGs) were recorded by inserting bipolar electrodes into the three limbs (Electrophysiological Recording System – Acquii2, Cartesian Labs, Toronto, ON). Two subcutaneous 6 cm² titanium mesh-patch electrodes were placed on the left and right chest wall laterally for defibrillation. Normothermia was maintained using a humidifier (Bourns Medical Systems Inc., Riverside, CA) to heat inspired gas to 38°C. All animals were treated with heparin (100 IU/kg IV) as a single bolus once catheters were in place. Rotigaptide was administered through the right jugular vein.

### Electrophysiology Studies

PR interval, QRS duration, and QT interval were measured after a 20-min baseline period. One min after constant pacing at 240-millisecond cycle length, ventricular effective refractory period (VERP) to the nearest 2 ms was determined by the premature stimulus method (an S₂ stimulus was delivered following 8 beat pacing trains at 240 millisecond cycle length). MAP duration at 90% repolarization (MAPD90) was measured from the right ventricular endocardial MAP catheter signal. All electrophysiological measures were performed at baseline, after drug or saline infusion, and averaged over 5 consecutive beats.

### Drug Administration

Once the surgical procedures were complete with stable blood pressure and adequate anesthesia, rotigaptide (ZP123, Wyeth Pharmaceuticals, Madison, NJ) or saline were given as an intravenous bolus loading dose (1.5 µg/kg) followed by a 15-min intravenous infusion (94 ng/kg/min) by a pump (Harvard Apparatus, Holliston, MS). The rabbits were randomly assigned to receive either rotigaptide or saline (n = 8 in each of 4 groups: rotigaptide 300 V protocol group, rotigaptide 500 V protocol group, saline 300 V protocol group and saline 500 V protocol group).

- A venous blood sample was obtained at the end of each experiment for plasma drug concentration measurement.

### Cardiac Arrest and Defibrillation Protocols

VF was induced using 2 seconds of 10 V of fully rectified 60 Hz current via the MAP catheter using a high voltage stimulator (HVS-02; Ventritex, Inc., Sunnyvale, CA) post saline or rotigaptide infusion. The endotracheal tube was immediately disconnected from the mechanical ventilator at the beginning of VF.
to simulate cardiac arrest without cardiac pulmonary resuscitation (CPR) for 4 min. Cardiac arrest was defined by identification of ventricular fibrillation (VF) on ECG and sudden absence of arterial systolic pressure.

- Following 4 min of untreated VF, 10 ms total pulse duration, biphasic defibrillation shocks were delivered using an external defibrillator (HVS-02; Ventritex, Inc., Sunnyvale, CA) with initial shock voltage starting at 300 V (saline, n = 8 and rotigaptide, n = 8) and 500 V (saline, n = 8 and rotigaptide, n = 8). If the first shock failed to defibrillate, subsequent shock voltage was increased by 200 V increments (300 V → 500 V → 700 V → 900 V). Approximately 15 to 20 seconds elapsed between shocks to charge the defibrillator. Defibrillation energy requirements were defined as the lowest voltage value required to successfully defibrillate. To study if a higher starting energy, more likely to defibrillate with the first shock, would alter the results, the identical protocol was repeated, but starting with a 500 V initial shock. Total cumulative voltage corresponded to the sum of the defibrillation step values for each rabbit (during the 300 V or the 500 V protocol), until success value or the ultimate 900 V step was reached. In case of 900 V failure, 900 V shocks were repeated two times but the additional shocks were not used in the cumulative voltage calculation.

- Heart rhythm at 30 s, 1 min, and 3 min after the last successful defibrillation was recorded and classified as follows: supraventricular rhythm (SVR), ventricular rhythm (VR < 30 bpm), asystole and slow ventricular rhythm ≤ 30 bpm (AS).

Immunohistochemistry

After the defibrillation protocol was completed, hearts were quickly excised, sectioned and rinsed with 0.01 M phosphate-buffered saline (PBS). Samples of the left ventricle from vehicle-treated and rotigaptide-treated rabbits were fixed in 4% paraformaldehyde in 0.01 M PBS overnight and then transferred to cryoprotectant solution of 30% sucrose (Sakura Finetek, Torrance, CA) and frozen at -30°C. Using a Leica HM500 (Bannockburn, IL) cryostat microtome, 10-µm sections were cut and allowed to dry on Superfrost Plus slides (Surgipath, Richmond, IL) at 57°C for 20 min. Sections were washed with 0.2% Triton X-100 for 30 min and then incubated with blocking solution (20% normal donkey serum (NDS)/1% bovine serum albumin/1% H2O2 in PBS) for 20 min. Sections were incubated overnight at 4°C with mouse anti-rabbit connexin 43 (MAB3068; Chemicon, Temecula, CA) diluted 1:400 in 1% NDS. The sections were washed, incubated with biotinylated donkey anti-rabbit serum (1:1000 in 1% NDS; Jackson Labs, Bar Harbor, ME), and the immunoreactivity visualized with a standard avidin-biotin complex (VECTASTAIN® Elite ABC kit; Vector Labs, Burlingame, CA) method. After incubation with 3,3'-diaminobenzidine (DAB), sections were transferred to TRIS, dehydrated and coverslipped with Permount. Immunolabeled tissue sections were visualized using a Nikon light microscope.

**Statistical Analysis**

Continuous variables were expressed as mean ± standard deviation (SD) and compared using the unpaired Student t test, the Fisher’s exact test, and the Wilcoxon two-sample test. A P value less than .05 was considered statistically significant. The relationship between successful defibrillation and shock energy was compared in the treatment and control groups using logistic regression.

**Results**

The effects of rotigaptide or saline on electrophysiological parameters are shown in Table 1. There were no significant differences before versus after saline, or before versus after rotigaptide, or between saline and rotigaptide, for any of the following parameters: heart rate, PR interval, QRS duration, QT interval, heart rate corrected QT interval (QTc), effective refractory period or mean action potential duration at 90% repolarization.

- Using a stepwise increase in defibrillation voltage, rotigaptide-treated animals were successfully defibrillated at lower voltages than saline-treated rabbits (Table 2). In the rotigaptide group, all animals demonstrated successful defibrillation after the 700 V defibrillation step during the 300 V protocol and after 900 V during the 500 V protocol. Four of the 16 saline-treated animals exhibited refractory VF (one in the 300 V protocol and 3 during the 500 V protocol), and could not be defibrillated even after 3 successive shocks at 900 V.
The median successful voltage for the rotigaptide-treated rabbits was 500 V versus 700 V in the control group \( (P = .0015) \). The average cumulative voltage of all shocks was significantly reduced during both the 300 V and the 500 V protocol in rotigaptide-treated rabbits compared to saline-treated animals (Table 1). Figure 1 shows the cumulative percentage of successful shocks at

Table 1. Effect of ZP123 or Saline on Electrophysiological Parameters

<table>
<thead>
<tr>
<th>EP Variables</th>
<th>Before Saline</th>
<th>After Saline</th>
<th>Before Rotigaptide</th>
<th>After Rotigaptide</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>300 V Protocol</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>252.5 ± 11.6</td>
<td>253.9 ± 14.5</td>
<td>257.4 ± 27.6</td>
<td>261.9 ± 34.2</td>
</tr>
<tr>
<td>PR (ms)</td>
<td>77.6 ± 7.6</td>
<td>80.6 ± 8.5</td>
<td>76.0 ± 21.6</td>
<td>74.9 ± 21.3</td>
</tr>
<tr>
<td>QRS (ms)</td>
<td>45.6 ± 6.5</td>
<td>46.4 ± 6.1</td>
<td>45.3 ± 7.1</td>
<td>45.8 ± 6.3</td>
</tr>
<tr>
<td>QT (ms)</td>
<td>157.1 ± 8.5</td>
<td>155.0 ± 7.6</td>
<td>156.8 ± 9.3</td>
<td>162.5 ± 20.0</td>
</tr>
<tr>
<td>ERP (ms)</td>
<td>97.5 ± 11.0</td>
<td>97.4 ± 5.7</td>
<td>102.3 ± 14.6</td>
<td>102.1 ± 14.1</td>
</tr>
<tr>
<td>MAPD90 (ms)</td>
<td>127.4 ± 18.8</td>
<td>125.0 ± 19.1</td>
<td>129.3 ± 19.4</td>
<td>130.0 ± 18.7</td>
</tr>
</tbody>
</table>

| **500 V Protocol** | | | | |
| HR (bpm) | 229.1 ± 17.0 | 230.1 ± 16.1 | 234.9 ± 26.5 | 233.2 ± 28.4 |
| PR (ms) | 78.6 ± 6.3 | 79.5 ± 7.2 | 77.5 ± 9.1 | 77.3 ± 9.5 |
| QRS (ms) | 45.8 ± 6.9 | 46.0 ± 6.8 | 40.0 ± 2.7 | 39.1 ± 2.3 |
| QT (ms) | 168.6 ± 14.6 | 166.1 ± 11.0 | 161.8 ± 12.4 | 162.9 ± 14.2 |
| ERP (ms) | 100.0 ± 6.8 | 102.6 ± 10.0 | 98.4 ± 11.1 | 99.6 ± 9.6 |
| MAPD90 (ms) | 137.7 ± 27.9 | 138.1 ± 27.4 | 130.3 ± 14.8 | 125.3 ± 12.8 |

Note: Values represent mean ± SD for n = 8 animals per group. HR: heart rate, bpm: beats per minute, PR: PR interval, QRS: QRS duration, QT: QT interval, ERP: effective refractory period, MAPD90: mean action potential duration at 90% repolarization. There were no significant differences between before versus after saline, before versus after rotigaptide, between saline and rotigaptide for either 300 V or 500 V protocol.

Table 2. Effect of Saline or Rotigaptide on Defibrillation Threshold in Rabbit Hearts Following Induction of Ventricular Fibrillation

<table>
<thead>
<tr>
<th>Defibrillation Voltage</th>
<th>Saline-Treated</th>
<th>Rotigaptide-Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>300 V Protocol</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>300 V</td>
<td>2/8 (25%)</td>
<td>4/8 (50%)</td>
</tr>
<tr>
<td>500 V</td>
<td>2/6 (33.3%)</td>
<td>2/4 (50%)</td>
</tr>
<tr>
<td>700 V</td>
<td>3/4 (75%)</td>
<td>2/2 (100%)</td>
</tr>
<tr>
<td>900 V</td>
<td>0/1 (0%)</td>
<td>N/A</td>
</tr>
<tr>
<td>Mean Cumulative Voltage</td>
<td>1137.5 ± 719</td>
<td>725.0 ± 525.8 *</td>
</tr>
</tbody>
</table>

| **500 V Protocol**     |               |                     |
| 500 V                  | 3/8 (37.5%)   | 4/8 (50%)           |
| 700 V                  | 2/5 (40%)     | 3/4 (75%)           |
| 900 V                  | 0/3 (0%)      | 1/1 (100%)          |
| Mean Cumulative Voltage | 1275.0 ± 742.1 | 962.5 ± 575.5 \*    |
| All protocols          | 12/16         | 16/16               |
| Median Success Voltage | 700           | 500 \*              |
| Mean Cumulative Voltage | 1206 ± 709   | 844 ± 546 \*        |

Note: Values represent mean ± SD for n = 8 animals per voltage protocol. The number of animals tested at each voltage is the denominator; e.g. 2/8 animals in the saline group 300 V protocol were defibrillated at 300 V; 6 were thus tested at 500 V and so on. Mean successful voltage cannot be calculated for the saline-treated rabbits since 4 in all could not be defibrillated at any tested voltage. Mean cumulative voltage for rotigaptide-treated rabbits vs. saline-treated rabbits were compared using the unpaired Student \( t \) test. Median success voltage was tested using the signed rank test.

\*\( P < .05 \), rotigaptide-treated rabbits vs. saline-treated rabbits.
every voltage, including all defibrillation attempts, for the 2 groups. Rotigaptide-treated rabbits were more likely to be successfully defibrillated at all voltages ($P < .05$).

- The heart rhythm at 30 s, 1 min, and 3 min after the last successful defibrillation was analyzed and listed in Figure 2. Three heart rhythm groups were defined: supraventricular rhythm, ventricular rhythm ($>30$ bpm), and asystole + slow ventricular rhythm ($\leq 30$ bpm). Most of rotigaptide-treated rabbits were in supraventricular rhythm at each step ($P < .05$).

Table 3. Effect of Rotigaptide or Saline on Systemic Hemodynamic Parameters 30 S and 1 Min After Last Successful Defibrillation

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Parameters</th>
<th>ASP 30 s</th>
<th>ASP 1 min</th>
<th>ADP 30 s</th>
<th>ADP 1 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>300 V</td>
<td>Mean ± SD</td>
<td>2.8 ± 4.1</td>
<td>1 ± 2.8</td>
<td>1.6 ± 3.0</td>
<td>0.7 ± 2.1</td>
</tr>
<tr>
<td>Saline</td>
<td>Pressure &gt;0 (%)</td>
<td>37.5</td>
<td>12.5</td>
<td>25</td>
<td>12.5</td>
</tr>
<tr>
<td>300 V</td>
<td>Mean ± SD</td>
<td>16.2 ± 6.3**</td>
<td>12.2 ± 7.6**</td>
<td>12.0 ± 5.1**</td>
<td>9.9 ± 6.1**</td>
</tr>
<tr>
<td>Rotigaptide</td>
<td>Pressure &gt;0 (%)</td>
<td>100*</td>
<td>87.5*</td>
<td>100*</td>
<td>87.5*</td>
</tr>
<tr>
<td>500 V</td>
<td>Mean ± SD</td>
<td>4.8 ± 6.8</td>
<td>2.2 ± 4.2</td>
<td>2.9 ± 4.0</td>
<td>1.9 ± 3.5</td>
</tr>
<tr>
<td>Saline</td>
<td>Pressure &gt;0 (%)</td>
<td>37.5</td>
<td>25</td>
<td>37.5</td>
<td>25</td>
</tr>
<tr>
<td>500 V</td>
<td>Mean ± SD</td>
<td>15.3 ± 10.1**</td>
<td>12.3 ± 9.1**</td>
<td>11.5 ± 7.9**</td>
<td>10.1 ± 7.3**</td>
</tr>
<tr>
<td>Rotigaptide</td>
<td>Pressure &gt;0 (%)</td>
<td>75</td>
<td>75</td>
<td>75</td>
<td>75</td>
</tr>
</tbody>
</table>

Note: Most values in saline group were zero; responses are dichotomized as either 0 or >0 and compared with the Fisher’s exact test and the Wilcoxon Two Sample test.

ASP: Aortic systolic pressure (mmHg), ADP: aortic diastolic pressure (mmHg). Percentage of positive values (pressure > 0 in %), standard deviation (SD).

* $P < .05$, rotigaptide-treated rabbits vs. saline-treated rabbits within each protocol.

** $P < .05$, rotigaptide-treated rabbits vs. saline-treated rabbits within each protocol.

Figure 1. Cumulative percentage of successful shocks at every voltage, including all defibrillation attempts, for the two groups. We compared the probability of successful defibrillation at all voltages between rotigaptide and control animals using logistic regression. Rotigaptide-treated rabbits were more likely to be successfully defibrillated at all voltages ($P < .05$).

Figure 2. Heart rhythm 30 s, 1 min and 3 min after the last successful defibrillation. SVR: supra-ventricular rhythm, VR: ventricular rhythm ($>30$ bpm), AS: asystole + slow ventricular rhythm ($\leq 30$ bpm). Fisher exact tests were used to compare rotigaptide-treated rabbits vs. saline-treated rabbits for each rhythm within each period time ($P < 0.05$).
parameters during the baseline state. Rotigaptide has no effect on electrophysiological improvement post shock cardiac rhythm and blood pressure. Following defibrillation, rotigaptide-treated rabbits have voltage requirements following 4 min of untreated VF. The main findings of the present study are that rotigaptide administration significantly decreases defibrillation energy requirements following 4 min of untreated VF. Following defibrillation, rotigaptide-treated rabbits have improved post shock cardiac rhythm and blood pressure. Rotigaptide has no effect on electrophysiological parameters during the baseline state.

- Myocardial ischemia is known to make defibrillation more difficult by increasing spatial electrical heterogeneity (evidenced by regional conduction velocity slowing), increasing conduction velocity dispersion, and increasing dispersion in VF cycle length. Hypoxia, ischemia, and acidosis induce intercellular gap junction uncoupling, and may contribute to conduction slowing and wavelet fragmentation during VF. Three factors are involved in conduction velocity: the electrical coupling between myocytes mediated by gap junctions; the sodium channels, which are the major channel proteins involved in excitability; and the tissue architecture (cell disposition, fiber shape, and interstitial collagen content). There have been few studies testing acutely administered drugs during or just before VF in cardiac arrest models. Regional conduction velocity slowing induced by sodium channel blockers administered just before or during VF increases defibrillation thresholds, whereas K⁺ blockers usually decrease defibrillation threshold.
- Rotigaptide (ZP123) is an antiarrhythmic peptide that preserves intercellular coupling during acidosis and diminishes conduction velocity slowing, eliminating the arrhythmogenic substrate in a Langendorff-perfused guinea pig heart model, without any effect on membrane currents (especially sodium channels).
- Previous studies have shown that regional gap junction inhibition increases the energy needed to successfully defibrillate, whereas global gap junction inhibition decreases DFT values. The effect of gap junction “openers” on DFT has not previously been tested. The fact that either gap junction blockers or gap junction “openers” can decrease defibrillation thresholds, seems to be contradictory. However, the models involved in these two studies were completely different: 16-doxyl-stearic acid (16-DSA), a gap junction blocker, decreases defibrillation energy requirements after brief VF (15 seconds) in Langendorff-perfused and therefore not substrate depleted (ie, not ischemic) isolated rabbit hearts. In contrast, the current study used a more clinically relevant in vivo rabbit model of longer lastingVF (4 min), presumably allowing enough time for ischemia-mediated electrophysiologic and metabolic changes, including conduction slowing, to become manifest.
- Xing and colleagues have recently shown that the preventive effect of rotigaptide on re-entrant VT (in an open chest dog model after coronary artery occlusion), was closely correlated to reversal of functional unidirectional conduction block. After infarction, the closure of gap junction channels is associated with a non-uniform downregulation of connexin 43, which may lead to unidirectional conduction block and re-entrant circuits. Gap junctional remodeling induced by ischemia is related to dephosphorylation of Cx43 within gap junctions and translocation of Cx43 from gap junctions to intracellular sites. Although the precise mechanism of action of rotigaptide is not precisely known, it may act by preventing connexin dephosphorylation, and thus preserving connexin function under conditions of metabolic stress. Because cardiac arrest in humans may often occur in the presence of myocardial ischemia, it is possible that enhancing gap junction conductance in ischemic VF may have a larger effect than that observed in the current model. The first changes in the amount and distribution of phosphorylated and non-phosphorylated isoforms of connexin 43 (obtained by immunoblotting and confocal immunofluorescence microscopy), appear after 7 min of global ischemia in an adult rat heart Langendorff model. However, myocardial oxygen consumption during VF is approximately twice that during the normally beating state, likely attributable to the higher contraction frequency.
suggestion that a gap junction opener can alter defibrillation after as little as 4 to 5 min of global ischemia during VF. Given that a marked reduction in gap junctions is required to result in cellular uncoupling, and the relatively short duration of VF compared to the time required for gap junction mediated uncoupling in other models, the mechanism of rotigaptide-related changes in defibrillation voltage are unexplained. Similarly, rotigaptide reduces infarct size in a rat model, but the mechanism has not been elucidated.17

- Several studies have evaluated the dose response effect of rotigaptide after bolus plus infusion intravenous dosing. Xing and colleagues28 administered rotigaptide as an 11 µg/kg bolus plus 1.2 µg/kg/hr infusion and tested its efficacy in prevention of canine ventricular tachycardia. In a second canine study, rotigaptide was administered as a 1 µg/kg bolus, plus 10 µg/kg/hr infusion and provided significant reduction in reperfusion arrhythmias.24 In a third canine study, rotigaptide was administered as up to a 45 µg/kg bolus and 42 µg/kg/hr infusion (resulting in concentrations of 181 nM/L), with efficacy against AF inducibility.19 Based on these results and exposures, we chose the 1.5 µg/kg bolus plus 5.6 µg/kg/hr infusion doses for the rabbit defibrillation threshold studies. We did not perform dose-response studies, and it is possible that lower doses and concentrations of rotigaptide may have had similar effects. At concentrations of 1.0, 7.7, and 69.2 nM, rotigaptide prevented VT inducibility in 6/12, 7/13, and 9/13 open chest dogs with infarction, respectively.13

- Likely due to the short period of ischemia in our study, we did not observe any changes in the distribution or amount of Cx43 along the membranes between the different groups of rabbits; possibly the immunohistochemistry assessments were not sensitive enough to detect these changes. In a recent study, neither the overall expression level of Cx43 (Western blotting) nor the spatial localization of this protein was altered after exposure of cells to rotigaptide for 5 hours.30

Electrophysiological Effects of Rotigaptide

As previously documented, rotigaptide had no effect on average heart rate, PR interval, QT interval, QRS duration, MAPD90, or ERP.14 These findings are consistent with the fact that rotigaptide has no effect on membrane currents and no effect on cellular repolarization.19

Limitations

This study was performed in normal rabbits and may not apply to diseased and/or larger hearts, such as in humans.31 Nevertheless, human VF appears more commonly during ischemia in failing myocardium than in healthy hearts, and the degree of heart failure is significantly correlated with earlier uncoupling after ischemia.32

- Another limitation is that rotigaptide was infused before VF, whereas in the clinical setting it would potentially be given many minutes after VF onset. Because this was a short-term study, we have no information on long-term survival rates after rotigaptide in this model of untreated VF.
- A previous study in isolated rabbit hearts has shown that rotigaptide exerts its effect on gap junction intercellular conductance and reduces epicardial anisotropy via activation of protein kinase C (PKC).30,33 PKC activation is known to reduce the cardiac sodium current peak and the myocardial excitability.34 Although the effect of rotigaptide in our study may have been mediated by an effect on PKC, sodium channel block by protein kinase C would be expected to increase rather than reduce DFT.5 This study is not able to define the mechanism of rotigaptide effect in this in vivo model, as the assessment of cellular coupling during VF is not possible. Further studies are needed to investigate the effect of rotigaptide on activation patterns during VF as well as after the shock. Optical and electrical mapping studies could be the next step to better understand how this drug improves defibrillation efficacy.

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References


