Effects of the Gap Junction Modifier Rotigaptide (ZP123) on Atrial Conduction and Vulnerability to Atrial Fibrillation

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Background—Altered conduction is associated with increased atrial fibrillation (AF) vulnerability in canine models of chronic mitral regurgitation (MR) and heart failure (HF). Rotigaptide (ZP123) augments gap junction conductance, improving cell-to-cell coupling. We studied the effects of rotigaptide on atrial conduction and AF vulnerability in the canine MR and HF models.

Methods and Results—Twenty-one dogs in 3 groups were studied: control (n=7), chronic MR induced by mitral avulsion (n=7), and HF induced by ventricular tachypacing (n=7). Epicardial mapping of both atria was performed with a 512-electrode array at baseline and at increasing rotigaptide doses (10, 50, and 200 nmol/L). Conduction velocity increased in both atria in control animals and MR animals (maximum percentage increase: 24±5%, 38±6% [P<0.001, <0.001] in the left atrium and 19±9%, 18±3% [P<0.001, <0.001] in the right atrium). Conduction velocity did not change in the left atrium of the HF group and increased minimally in the right atrium (3±3%, 17±5% [P=NS, P=0.001]). AF duration was increased at baseline in MR and HF animals (control: 16±25 seconds, MR: 786±764 seconds, HF: 883±684 seconds; P=0.013). At 50 nmol/L of rotigaptide, duration of AF markedly decreased in the MR animals (96% reduction, P<0.001), reducing AF duration to that of control animals (control: 9±11 seconds, MR: 14±16 seconds, HF: 1622±355 seconds; P=0.04).

Conclusions—Gap junction modulation with rotigaptide reduces AF vulnerability in a canine MR model of AF to a level similar to control animals but does not affect AF vulnerability in the canine HF model. This may be a novel therapeutic target in some forms of AF. (Circulation. 2006;114:110-118.)

Key Words: atrium ■ fibrillation ■ electrophysiology ■ conduction

Atrial fibrillation (AF) is a common clinical arrhythmia that is usually associated with hypertension, heart failure, or mitral valve disease, but it can also occur in the absence of these diseases.1 Several electrophysiological substrates have been shown to increase vulnerability to AF.2,3 Although shortening of atrial refractoriness has been implicated as the electrophysiological substrate of AF vulnerability in atrial tachycardia and methacholine models of AF,4,5 abnormalities in conduction are crucial in models of heart failure (HF)6 and chronic mitral regurgitation (MR).7,7

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Myocyte cell-to-cell coupling through gap junctions is a key factor in arrhythmogenesis in a variety of conditions.8–10 Rotigaptide (ZP123) is a hexapeptide that specifically augments gap junction conductance, improving cell-to-cell coupling11–14 without any atrial or ventricular proarrhythmic effects.11,15,16

The present study investigates the effects of rotigaptide on atrial electrophysiology in normal and diseased atria in models of AF in which conduction abnormalities exist (HF and MR). The study tests the hypothesis that enhancement in atrial conduction decreases the vulnerability to AF.

Methods

All animal studies were performed according to National Institute of Health guidelines and approved and monitored by the Laboratory Animal Resource Center’s Institutional Animal Care and Use Committee at University of California San Francisco. Twenty-one dogs (25 to 30 kg) were divided into 3 groups: control (n=7), MR (n=7), and HF (n=7). For all procedures, isoflurane anesthesia was used, and the animals were mechanically ventilated after intubation.

Surgical Procedure for the MR Model

The MR model of AF has been previously described in detail.7 In brief, the mitral chordae tendineae were avulsed through a transvenous approach until moderate to severe MR was achieved on transesophageal echocardiography. Six weeks after creation of MR, the animals were subjected to an open-chest mapping and electrophysiology (EP) study (see below).

Surgical Procedure for the HF Model

HF was produced by ventricular tachypacing, as previously described.6 A permanent pacemaker was implanted and programmed to

Received December 5, 2005; revision received May 3, 2006; accepted May 5, 2006.
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Circulation is available at http://www.circulationaha.org
DOI: 10.1161/CIRCULATIONAHA.105.606251
pace at a rate of 240 bpm at an output of 4 times the threshold. Animals in this group underwent an open-chest follow-up study after 4 weeks of pacing.

**Monitoring of the MR and HF Models**

All dogs were subjected to weekly physical examinations and transthoracic echocardiography in the 4-chamber view to measure left atrial (LA) size and left ventricular (LV) function. HF was established by clinical signs (lethargy, edema, and mucous membrane color), and LV function determined by echocardiography. No signs of HF or depression of LV function were observed at any time in the control and MR dogs.

**Open-Chest Follow-Up Studies**

At follow-up, dogs were anesthetized with isoflurane, and the chest was opened with a midline sternotomy. A pericardial cradle was created, and epicardial multielectrode plaques were placed on the free wall of the right atrium (RA) and LA, with a total of 512 electrodes (interelectrode distance=3.5 mm), as previously described.17 Unipolar electrode signals were simultaneously digitized at 2-kHz sampling with the UnEmap system (Auckland Uniservices Ltd, Auckland, New Zealand). Neighboring pairs of electrodes on the plaques were also used for bipolar stimulation at an amplitude of twice diastolic threshold. Effective refractory periods (ERP) were measured at 6 LA and 6 RA sites with the $S_1-S_2$ method, where $S_2$ was incremented in 2-ms steps after 8-beat drive trains with basic cycle lengths (BCLs) of 200, 300, and 400 ms. Dispersion of repolarization at each atrium was expressed as the coefficient of variation of the ERP (SD/mean). At the same BCL, during stimulation of the contralateral atrium, conduction vectors and conduction velocities (CVs) were calculated at each recording site on the basis of activation data using an algorithm previously described.18 Phase differences, calculated as the average difference with neighboring activation times at each site, were also measured to quantify the spatial heterogeneity of conduction, as previously described.6,19 Frequency histograms were constructed for the phase differences within a recorded area. These histograms were summarized as the median phase time P50, and the 5th and 95th percentiles, P5 and P95, of the distribution. The absolute degree of heterogeneity was quantified as the width of the distribution, P95–P5. AF inducibility was tested by burst pacing at a 50-ms BCL at 1 LA and 1 RA site; six 6-second and six 12-second bursts were applied at each site. In each animal, the duration of each episode of AF was measured; AF was considered to be sustained when an episode lasted >30 minutes.

In addition, 2 plunge electrodes were placed at the anterior wall of the left ventricle for programmed stimulation. Pacing was performed at twice diastolic threshold. ERP's were calculated by using the method described for the atrium at 3 BCLs (250, 300, and 350 ms). At the end of the study, the (highest rotigaptide dose), ventricular programmed stimulation with 2 BCLs (300 and 350 ms) and up to 3 extrastimuli was performed to evaluate the inducibility of ventricular arrhythmias.

All measurements were performed at baseline and repeated at 3 escalating doses of rotigaptide calculated to provide estimated serum concentrations of 10, 50, and 200 nmol/L, based on the compound’s pharmacokinetics. On the basis of these calculations, the compound was infused intravenously with a bolus of 1.5, 15, and 45 µg/kg, respectively, followed by a continuous infusion of 1.4, 14, and 42 µg/kg per hour. Because the compound is expected to reach steady state within 15 minutes, all measurements were performed 20 minutes after the start of the infusion at each dose.

**Histology and Immunohistochemistry**

Transmural tissue samples from the LA and RA appendage and free wall were fixed in 10% neutral buffered formalin solution. Tissue was processed, embedded in paraffin, and sectioned in 4- to 5-µm-thick sections. Serial sections were stained with hematoxylin and eosin, Masson’s trichrome, or Sirius red.

Immunohistochemistry analysis for connexin 40 and connexin 43 was also performed in tissue samples from the LA and RA appendage and free wall as previously described.21 Using rabbit polyclonal anti-Cx40 (Alpha Diagnostics Int, San Antonio, Tex) or anti-Cx43 (Chemicon Int, Temecula, Calif), mouse monoclonal antisense antibody (DAKO Denmark A/S, Glostrup, Denmark). To compare the distributions of Cx40 and Cx43, sections were labeled with a combination of rabbit polyclonal anti-Cx40 antibody and mouse monoclonal anti-Cx43 (BD Biosciences, San Jose, Calif). For fibrosis quantification, sections were labeled with a combination of anticalcine fluorescence and anti-desmin antibody. The green pixel content of digitized images (collagen labeling) relative to the total tissue area was counted by using the Adobe Photoshop 7.0 software package, as previously described.3

**Statistical Analysis**

All values are reported as mean±SD (except for figures that show mean±SEM), unless mentioned otherwise. Repeated-measures ANOVA, with Huynh-Feldt correction when appropriate, was performed to evaluate the effect of the increasing doses of rotigaptide in each group. Within-groups effect was calculated by the estimated marginal means method with the Bonferroni correction for multiple comparisons. Comparisons among groups were made by using 1-way ANOVA. Once statistical difference among groups was established, between-groups differences were tested with the Scheffé test. For AF vulnerability, nonparametric tests were used: the Friedman test to evaluate the effect on each group of increasing concentrations of rotigaptide and the Kruskal-Wallis H test for the differences between groups at each dose. Statistical significance was defined as P<0.05.

The authors had full access to the data and take full responsibility for its integrity. All authors have read and agree to the manuscript as written.

**Results**

All animals were in normal sinus rhythm at the beginning of the final follow-up study. Two HF dogs died during induction of anesthesia at the time of the follow-up, before administering rotigaptide and the start of any EP data measurements due to hemodynamic instability. All other animals were hemodynamically stable with normal oxygen blood saturation during the entire procedure. The central temperature was kept stable at 35°C. Heart rate increased slightly with rotigaptide (in a dose-dependent fashion) in all groups without significant changes in blood pressure in any group or at any dose (see the Table).

**Atrial Refractoriness**

The average ERP for the left and right atria are plotted for each dose of rotigaptide at the 3 tested BCLs in Figure 1. In the LA of control animals, rotigaptide administration resulted in a small shortening of ERP from 105±1 to 98±2 ms at a 200-ms BCL (P=0.093), from 113±1 to 102±2 ms at a 300-ms BCL (P<0.001), and from 115±2 to 101±2 ms at a 400-ms BCL (P=0.012). A similar pattern was seen in HF dogs, with a decrease in ERP from 111±5 to 106±4 ms at a 200-ms BCL (P=0.02), from 118±4 to 106±4 ms at a 300-ms BCL (P<0.001), and from 120±5 to 105±6 ms at a 400-ms BCL (P<0.001). The MR group response was similar but did not reach statistical significance. The degree of shortening of the
ERP was alike for all 3 groups at each dose and BCL. In contrast, RA ERP did not change for any group with the administration of rotigaptide.

Rotigaptide administration had no effect on rate adaptivity of the ERP, which was preserved in each group and at each dose of rotigaptide, as can be seen in Figure 1.

Dispersion of repolarization was quantified as the coefficient of variation (COV) of the ERP (SD/mean). At baseline, there was a significant difference between the models, with the MR and HF groups having a higher COV of ERPs than control in the LA. Although the control group still had lower COV of ERPs with rotigaptide, the difference between the other groups at each dose was not significant (Figure 2).

**Atrial Conduction Velocity**

At baseline (before rotigaptide), CV was not statistically different between the groups at any paced cycle length (Figure 3). However, CV tended to be slower in the MR and HF groups compared with the control group.

In the control group, CV in the LA significantly increased with respect to baseline by 28±3% at a 200-ms BCL, 24±5% at a 300-ms BCL, and 24±7% at a 400-ms BCL with the administration of rotigaptide (P=0.001, <0.001, and 0.015 at 200-, 300-, and 400-ms BCLs, respectively) (Figure 3). In the RA, CV also increased with rotigaptide, by 25±7% at a 200-ms BCL, 19±9% at a 300-ms BCL, and 15±7% at a 400-ms BCL (P=0.007, <0.001, and 0.121 at 200-, 300-, and 400-ms BCLs, respectively).

Conduction velocity in the MR group also increased with respect to baseline with rotigaptide in the LA by 35±7% at a 200-ms BCL, 38±6% at a 300-ms BCL, and 31±6% at a 400-ms BCL (P=0.001, <0.001, and 0.015). Similarly, CV in the RA increased by 18±4% at a 200-ms BCL, 18±3% at a 300-ms BCL, and 21±7% at a 400-ms BCL (P=0.001, <0.001, and 0.006), albeit to a lesser degree than in the LA.

In the HF group, CV did not increase with rotigaptide in the LA and had only a modest increase in the RA, with an increase of 17±5% at a 300-ms BCL and 20±8% at a 400-ms BCL (P=0.003 and P=0.055, respectively). This increase was significantly smaller than that of the control and MR groups. As a result, at the maximum rotigaptide dose LA CV in the HF group was significantly slower compared with that in the control or MR groups at all paced cycle lengths (P=0.001, <0.001, and 0.05 at 200-, 300-, and 400-ms BCLs, respectively).

Phase maps were constructed to measure the heterogeneity of conduction velocities. The absolute degree of heterogeneity of conduction velocities was increased by rotigaptide in all groups, with a more pronounced increase in the MR and HF groups compared with the control group. The phase maps in the control group showed a homogenous distribution of conduction velocities, whereas in the MR and HF groups, the phase maps showed a more heterogeneous distribution with larger areas of slow conduction.

**Hemodynamic Parameters for Each Group at Each Rotigaptide Dose During Follow-Up Study**

<table>
<thead>
<tr>
<th>Model</th>
<th>Baseline</th>
<th>10 nmol/L</th>
<th>50 nmol/L</th>
<th>200 nmol/L</th>
<th>P</th>
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<tr>
<td>Con (n=7)</td>
<td>HR</td>
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<td>131±14</td>
<td>129±12</td>
<td>128±11</td>
</tr>
<tr>
<td></td>
<td>SBP</td>
<td>103±24</td>
<td>88±21</td>
<td>96±14</td>
<td>104±14</td>
</tr>
<tr>
<td>MR (n=7)</td>
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<td>124±22</td>
<td>132±17</td>
<td>127±16</td>
</tr>
<tr>
<td></td>
<td>SBP</td>
<td>93±16</td>
<td>98±16</td>
<td>105±19</td>
<td>108±23</td>
</tr>
<tr>
<td>HF (n=5)</td>
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<td>126±42</td>
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<td>81±36</td>
<td>85±13</td>
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</tr>
</tbody>
</table>

Con indicates control; HR, heart rate in beats per minute; SBP, systolic blood pressure in mm Hg; and NS, no significant change. Data are provided as mean±SD.

Conduction velocity in the MR group also increased with respect to baseline with rotigaptide in the LA by 35±7% at a 200-ms BCL, 38±6% at a 300-ms BCL, and 31±6% at a 400-ms BCL (P=0.001, <0.001, and <0.001). Similarly, CV in the RA increased by 18±4% at a 200-ms BCL, 18±3% at a 300-ms BCL, and 21±7% at a 400-ms BCL (P=0.001, <0.001, and 0.006), albeit to a lesser degree than in the LA.

In the HF group, CV did not increase with rotigaptide in the LA and had only a modest increase in the RA, with an increase of 17±5% at a 300-ms BCL and 20±8% at a 400-ms BCL (P=0.003 and <0.001, respectively). This increase was significantly smaller than that of the control and MR groups.

Figure 1. Effect of increasing concentrations of rotigaptide on atrial refractoriness in open-chest studies. Average ERP for each group and driven BCL. Con indicates control. Con, n=7; MR, n=7; HF, n=5; data are mean±SEM. *,**Significant reduction of ERP with increasing concentration of rotigaptide. *P<0.001; **P<0.05.
ity is shown in Figure 4. At baseline, there was less heterogeneity of CV in the control group (0.38 ± 0.05 cm/s for LA, 0.88 ± 0.05 cm/s for RA) compared with the MR (0.66 ± 0.14 cm/s for LA, 1.05 ± 0.09 cm/s for RA) and HF (0.66 ± 0.06 for LA, 1.11 ± 0.12 for RA) groups in both atria at a 200-ms BCL. Similar findings were present at 300- and 400-ms BCLs. In the LA, CV heterogeneity did not substantially change with rotigaptide in the control group. In the HF group, there was only a small decrease in P95–P5 at a 300-ms BCL (P = 0.017); all other BCLs demonstrated no change in P95–P5 with rotigaptide. The MR group had the largest decrease in P95–P5, reaching statistical significance at 300- and 400-ms BCLs (P = 0.006 and P = 0.048, respectively). Therefore, at 50 nmol/L rotigaptide, there was no difference in LA P95–P5 between the MR and control groups at any paced BCL. No significant changes in CV heterogeneity were observed in any group in the RA.

Atrial Fibrillation Vulnerability

Atrial fibrillation vulnerability was measured on the basis of the longest episode of AF induced. Figure 5 plots the average longest induced AF episode for each group and dose. As expected, only brief episodes of AF were induced in the control group at baseline. There was no change in vulnerability in the control group with any concentration of rotigaptide. Thus, no AF proarrhythmia could be demonstrated with the use of the compound at any dose. As previously reported, there was an increase in AF vulnerability in the MR and HF models in the baseline state (control: 16 ± 25 seconds; MR: 786 ± 764 seconds; HF: 883 ± 684 seconds; P = 0.013). There was no difference in AF vulnerability in the baseline state (predrug) between the MR and HF groups. No effect on AF vulnerability at a 10-nmol/L dosing of rotigaptide was observed in any group. However, at 50 nmol/L, AF vulnerability in the MR dogs was significantly reduced (control: 9 ± 11 seconds; MR: 14 ± 16 seconds; HF: 1622 ± 355 seconds) to levels similar to those of the control animals, and no sustained AF episodes were observed. There was a 96 ± 1% reduction in AF duration in this group (P < 0.001). In the MR group, at a 200-nmol/L dosing of rotigaptide, AF vulnerability increased compared with that at 50 nmol/L (447 ± 538 seconds). In the HF group, none of the rotigaptide doses significantly affected AF vulnerability.

LV Stimulation

Limited studies in the left ventricle were conducted to determine if there was any significant ventricular proarrhythmia. At the 300-ms BCL, LV ERPs did not change with rotigaptide in any of the groups (control: 172 ± 10, 170 ± 6, 170 ± 9, and 169 ± 5 ms; MR: 164 ± 8, 163 ± 7, 167 ± 8, and 165 ± 13 ms; HF: 182 ± 12, 182 ± 13, 176 ± 12, and 173 ± 11 ms; for baseline, 10, 50, and 200 nmol/L, respectively). The same findings were present at the 250- and 350-ms BCLs. Ventricular programmed stimulation did not induce any ventricular arrhythmias in the control and MR groups. In contrast, a sustained monomorphic ventricular tachycardia with a cycle length of 200 ms was induced with a 300-ms BCL and 2 extrastimuli in 1 of the HF dogs, requiring ventricular burst pacing for termination.

Plasma Levels of the Compound

Plasma samples were drawn at each dose level in every animal. Plasma concentrations for each dosing were as follows: baseline, <1 ng/mL; low, 5.6 ± 6.0 nmol/L; middle, 56.2 ± 10.3 nmol/L; and high, 181.4 ± 39.6 nmol/L—close to the target concentrations of 10, 50, and 200 nmol/L.

Histology and Immunohistochemistry Studies

Transmural tissue sections from the RA and LA were stained with hematoxylin and eosin, Sirius red, or Masson’s

Figure 2. Effect of increasing concentrations of rotigaptide on dispersion of repolarization, expressed as the coefficient of variation of the ERP (C.o.V. ERP) for each driven BCL. Data are mean ± SEM. #Significant difference of MR dogs compared with control dogs (P < 0.04).
trichrome stain to compare tissue structure and the distribution of fibrous tissue in the 3 groups. Sections were also immunolabeled for fibrosis quantification (Figure 6). Both atria of the control group showed a normal histological structure. LA sections of MR dogs had a slight increase in fiber separation with some inflammatory infiltration. However, myocyte morphology was similar to that in the control dogs, without signs of cellular necrosis. The RA sections of the MR group showed no difference compared with the RA of the control dogs. In the LA of the HF group, marked interstitial fibrosis was present. These histological alterations were also seen in the RA, although they were less prominent. Fibrosis quantification showed a significant increase in percentage LA fibrosis in HF dogs compared with the control and MR models (7.7±1.1%, 8.0±2.8%, and 24.6±5.9% for the control, MR, and HF groups, respectively, \( P < 0.001 \)).

**Discussion**

The main findings of this study are that rotigaptide decreases AF vulnerability in a canine model of MR but not in HF. The compound concomitantly increased CV and decreased conduction heterogeneity in the LA of the MR dogs. CV change in the HF group was minimal.

We have previously shown that in the canine MR model, increased AF vulnerability is associated with direction-dependent atrial conduction abnormalities with decreased AF spatiotemporal organization.\(^3,7,22\) On the other hand, Li et al.\(^6,23\) have demonstrated that experimental HF promotes AF, causing changes in atrial ionic currents and extensive interstitial fibrosis that interferes with local conduction. A subse-
quent study showed that after recovery from experimental
HF, the ionic changes reverse but conduction abnormalities
and AF vulnerability remain. Histological studies and quan-
tification of tissue structural changes in these canine models
have been previously published. Consistent with previous
studies, the present study shows only a minimal increase in
interstitial fibrosis in the MR model with respect to the
control animals; however, there was marked fibrosis in the
HF model.

Chronic AF has been associated with a disturbance in the
distribution and/or change in the expression of connexin 40
and/or connexin 43 gap junction proteins in humans and in
animal models of AF. However, only limited studies have
been performed in those situations associated with an
increase in the susceptibility to AF, with variable results.
It is not known whether impairment in the number or function
of gap junction plays a role as a substrate predisposing to AF.
We show no apparent changes in the distribution or amount
of connexins between these models. Because disturbed con-
duction is a major feature of the MR and HF models, our data
suggest that these changes are due to fibrosis in the HF group
but may be more functional in nature in the MR group

because rotigaptide improved conduction and AF vulnerabil-
ity in the MR group but not in the HF group.

Rotigaptide has been previously shown to increase gap
junction conductance without affecting membrane cur-
rents. This effect is thought to be the mechanism respons-
ible for the prevention of reentrant ventricular tachycardias
during acute ischemia and acidosis-induced ventricular
conduction slowing in dogs. Haugan et al have demon-
strated that rotigaptide prevents atrial conduction slowing and
increases CV at the 100-nmol/L dose level during metabolic
stress in isolated normal LA from rats. These effects ap-
parently are also mediated by a selective effect on gap junction
intercellular communication with no effect in up to 80
cellular receptors and ion channels. A recent publication
studying the effects of rotigaptide on varying isoforms of
cconnexin demonstrate that rotigaptide is selective toward
Cx43. In addition, this study shows that the enhancement of
gap junctional communication is likely to be elicited by an
indirect route because rotigaptide did not modify the overall
level of Cx43 expression and changes in the phosphorylation
status of the protein were not observed. Our study is the first

Figure 4. Conduction heterogeneity.
Absolute phase heterogeneity (P95–P5) as a function of the concentration of roti-
gaptide for each studied driven BCL in the LA and RA. Data are mean ± SEM.
*Significant decrease in conduction heterogeneity with increasing concentrations
of rotigaptide (P < 0.05); #significant difference of the control group compared
with MR and HF groups (P < 0.01); ##significant difference of the control group
compared with the HF group (P < 0.04).

Figure 5. Effect of increasing concentrations of rotigaptide on
AF vulnerability in the control, chronic MR, and HF groups,
expressed as the average duration of the longest induced AF
episodes. Data are mean ± SEM, #Significant difference among
groups at each dose (P < 0.05); *significant reduction in AF dura-
tion across doses for the MR group compared with previous
doses (P < 0.001).

Figure 6. Tissue structure in control, MR, and HF left atria. First
column: Sirius red staining; middle column: anticallogen (green)
and antidesmin (red) immunofluorescence; last column: anticol-
lagen (green) and anti-Cx43 (red) immunofluorescence (repre-
sentative images at magnification ×400). In the HF group, fibro-
sis is prominent compared with control animals or MR animals.
to assess the in vivo effects of rotigaptide in normal and remodeled canine atria and its ability to prevent AF in these animal models. Our results show an effect on improving CV even in normal atria under physiological conditions. This difference observed in normal atria compared with normal ventricle (in which no CV effect is seen with rotigaptide) may be due to the marked nonuniform anisotropy that exists in the normal atrium and nonuniform distribution of connexin in the atrium (as compared with the ventricle).31,32

Although rotigaptide also improved conduction in the atria of the MR model, no effect in the HF model was observed. This further supports the idea of a different type of remodeling and hence, electrical substrate, in these 2 models.3,6,7,23 Interstitial fibrosis tends to be more prominent in the HF model. This probably results in a more profound and fixed uncoupling of cells and thus, a modest increase in gap junction conductance with rotigaptide may not have a meaningful effect on conduction. This may explain the lack of effect of the compound in the HF group. We have previously shown that the MR model has less fibrosis and that conduction heterogeneity and increased anisotropy appear to be more functional.3 This is confirmed in the present study, with the histology and immunohistochemistry showing significantly more collagen immunolabeling in the HF group. Previous studies in humans and animals25 have shown a redistribution in connexins in both models of chronic remodeling, although these changes are not consistent in all the studies.26,28 To date, studies of gap junctions in AF have focused on amount or distribution of connexins rather than on functionality. Our data further support the presence of a more severe anatomic derangement in the HF model as a substrate responsible for AF, as opposed to the MR model, in which the changes probably are more functional.

Our results show a consistent effect of rotigaptide on atrial ERPs at all tested BCLs and in all models. ERPs shortened in the LA in a similar fashion in all groups. Consistent with previous studies, rotigaptide did not change ventricular ERPs.12,16 How rotigaptide affects ERPs and why the effect is limited to the atrium are not well understood. Single-cell studies have demonstrated no effect of rotigaptide on action potential duration or characteristics12,13 and no binding of the compound to ion channels.13 Pacing thresholds remained constant during the study and were not affected by rotigaptide (data not shown). It is interesting to speculate that increasing gap junction conductance enhances electrotonic interactions, and, because the heterogeneity of action potential duration is greater in the atrium than the ventricle, this may affect ERP more in the atrium than in the ventricle or in isolated cells. An alternative explanation is that in tissue with more heterogeneous conduction at baseline (ie, atrium compared with the ventricle), increasing coupling may improve the safety factor for propagation, resulting in shorter measured ERPs without affecting action potential duration. Interestingly, we observed a differential effect of rotigaptide between the LA and the RA. This different effect was present in the control group as well as in the MR and HF groups. This finding would suggest that the differential effect of rotigaptide on LA and RA ERPs is related to the intrinsic structural properties of each atrium, perhaps as the result of the unique anatomy of the RA trabeculations. Because the ERPs were measured mostly on the RA free wall (trabeculated portions), improving coupling may not affect ERP as much as in the LA, where there are no trabeculations, and thus improving gap junction conductance probably have a larger effect on ERP. Further evidence of this is the fact that although CV increased in the RA with rotigaptide, the effect was significantly more modest than that in the LA.

Although rotigaptide resulted in ERP shortening, we did not observe any proarrhythmia. This was indicated by no significant change in AF duration or ease of inducibility. Wijffels et al15 have demonstrated in a previous report on the rapid atrial pacing model that ERP shortening has a direct

Figure 7. Connexin expression in the left atrium of control dogs, MR dogs, and HF dogs. A, Anti-Cx40 (green) and antidesmin (red) immunofluorescence and anti-Cx43 (green) and antidesmin (red) immunofluorescence (red). B, Anti-Cx40 (green) and anti-Cx43 (red) immunofluorescence (representative images at magnification ×400). Both connexins colocalized, and the amount and spatial distribution of the gap junction proteins did not appear to be markedly different between the groups.
effect on AF inducibility. Wijffels et al showed this effect with an average reduction on ERPs of 35%. At the same BCL, the average reduction on ERPs in the 3 groups in the present study was only 10%. Notably, in the Wijffels article and subsequent studies from that group, the development of sustained AF lagged behind the decrease in the ERP, suggesting that there was another “factor” contributing to the AF vulnerability besides ERP. On the other hand, improvement in conduction and conduction heterogeneity probably prevents fibrillatory conduction, wavebreaks, and possibly rotor anchoring, thereby reducing arrhythmia vulnerability.33

Atrial fibrillation inducibility showed marked differences between the groups. Consistent with previous reports,7,23 AF inducibility at baseline was significantly augmented in the MR and HF groups compared with control. More importantly, whereas in the HF group rotigaptide was not able to reduce AF vulnerability, in the MR dogs, AF inducibility was significantly reduced to the level of the control dogs at the 50-nmol/L dose of the compound. At the highest dose, vulnerability increased again in this group. A bell-shaped dose-response with rotigaptide had already been reported,13,20 and its mechanism is not known.

Limitations
The study compares the effects of rotigaptide in normal dogs and in 2 distinct models of atrial dilation. Whether the changes in atrial electrophysiology that occur in other models such as atrial tachycardia or vagal AF and hence AF vulnerability would be affected by rotigaptide is not known and was not the focus of this study. Only the acute effect of the compound was tested. The half-life of rotigaptide is very short, and no data are available with regard to its chronic use.

Another potential limitation is that the study was done in animals anesthetized with isoflurane, a compound known to be a partial gap junction uncoupler.34 However, the fact that rotigaptide appeared to have little or no effect in the RA of any model compared with the LA, or in either atrium in the HF group, suggests that the potential interaction between isoflurane and rotigaptide, if any, was only minor. Moreover, the dose of isoflurane was kept constant during the course of the study. Therefore, it is unlikely that isoflurane played a significant role in the results.

Many of the statistical tests failed to reach significance, probably due to low power because of the small size of the samples.

Conclusions
Gap junction modulation with rotigaptide improves atrial conduction in normal dogs and in the chronic MR canine model of AF. Rotigaptide reduces AF vulnerability in the MR model to the level of the control animals but did not change AF vulnerability in the HF model. These results suggest that therapies targeting gap junctions may be an effective antiarrhythmic approach in some forms of AF when functional conduction derangements exist.

Acknowledgments
We thank Kathleen Connell for her support in performing the plasma assays for the determination of the drug concentration.

Sources of Funding
This work was supported by National Heart, Lung, and Blood Institute grant RO1-HL072854 (Dr Olgin).

Disclosures
None.

References


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**CLINICAL PERSPECTIVE**

This study compares the effects of rotigaptide, a gap junction enhancer, in 2 different canine models of atrial dilation with increased vulnerability to atrial fibrillation (AF)—a heart failure model and a chronic mitral regurgitation model. These 2 models parallel common clinical situations in which AF often occurs. Rotigaptide is a derivative of a naturally occurring antiarrhythmic peptide that improves cell coupling, which is known to be a key factor in arrhythmogenesis. This study shows that this compound decreases AF vulnerability in the mitral regurgitation model but not in the heart failure group. These results demonstrate that functional alterations in gap junction conductance play a part in the substrate of some forms of AF. In addition, this study demonstrates the effect of a new class of antiarrhythmic drugs (drugs that increase gap junction conductance) on atrial electrophysiology and may be a novel treatment strategy for particular forms of AF.
Effects of the Gap Junction Modifier Rotigaptide (ZP123) on Atrial Conduction and Vulnerability to Atrial Fibrillation
Jose M. Guerra, Thomas H. Everett IV, Ken W. Lee, Emily Wilson and Jeffrey E. Olgin

*Circulation*, 2006;114:110-118; originally published online July 3, 2006;
doi: 10.1161/CIRCULATIONAHA.105.606251
*Circulation* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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