

# Effects of Chronic Gap Junction Conduction–Enhancing Antiarrhythmic Peptide GAP-134 Administration on Experimental Atrial Fibrillation in Dogs

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**Background**—Abnormal intercellular communication caused by connexin dysfunction may contribute to atrial fibrillation (AF). The present study assessed the effect of the gap junction conduction–enhancing antiarrhythmic peptide GAP-134 on AF inducibility and maintenance in a dog model of atrial cardiomyopathy.

**Methods and Results**—Twenty-four dogs subject to simultaneous atrioventricular pacing (220 bpm for 14 days) were randomly assigned to placebo treatment (PACED-CTRL; 12 dogs) or oral GAP-134 (2.9 mg/kg BID; PACED-GAP-134; 12 dogs) starting on day 0. UNPACED-CTRL (4 dogs) and UNPACED-GAP-134 (4 dogs) served as additional control groups. Change in left atrial (LA) systolic area from baseline to 14 days was calculated using transoesophageal echocardiography. At 14 days, animals underwent an open-chest electrophysiological study. PACED-CTRL dogs (versus UNPACED-CTRL) had a shorter estimated LA wavelength ( $8.0 \pm 1.4$  versus  $24.4 \pm 2.5$  cm,  $P < 0.05$ ) and a greater AF vulnerability (mean AF duration,  $1588 \pm 329$  versus  $25 \pm 34$  seconds,  $P < 0.05$ ). Oral GAP-134 had no effect on AF vulnerability in UNPACED dogs. Compared with PACED-CTRL dogs, PACED-GAP-134 dogs had a longer estimated LA wavelength ( $10.2 \pm 2.8$  versus  $8.0 \pm 1.4$  cm, respectively,  $P < 0.05$ ). Oral GAP-134 did not significantly reduce AF inducibility or maintenance in the entire group of 24 PACED dogs; in a subgroup of dogs ( $n = 11$ ) with less than 100% increase in LA systolic area, oral GAP-134 reduced AF induction from 100% to 40% and mean AF duration from  $1737 \pm 120$  to  $615 \pm 280$  seconds ( $P < 0.05$ ).

**Conclusions**—Oral GAP-134 reduces pacing-induced decrease in LA wavelength and appears to attenuate AF vulnerability in dogs with less atrial mechanical remodeling. Gap junction modulation may affect AF in some circumstances. (*Circ Arrhythmia Electrophysiol.* 2009;2:171-178.)

**Key Words:** atrial fibrillation ■ gap junctions ■ cardiomyopathy

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia, noted in approximately 20% of patients with heart failure. Its presence is associated with significant morbidity and mortality.<sup>1</sup> Antiarrhythmic drugs are only moderately effective at preventing recurrent episodes of AF<sup>2</sup> and have potentially severe side effects. New strategies to promote sinus rhythm in heart failure patients are required. Targeting underlying atrial substrates with novel mechanism-based treatments is a promising approach.<sup>3,4</sup>

Atrial myocytes are electrically coupled through gap junctions that are made up of proteins from the connexin family, which form connexons. Connexons localized at the intercalated disks bind head to head with other connexons from adjacent cells, forming a direct cytoplasmic continuity between the cells. These gap junctions are low-resistance

pathways for action potentials to spread from myocyte to myocyte. Clinical and experimental studies<sup>5,6</sup> have shown that cell–cell uncoupling is important in AF pathogenesis. Changes in connexin expression, phosphorylation, or distribution, as well as increased interstitial fibrosis (leading to “zigzag” propagation through an area of patchy fibrosis), may play a key role in promoting AF by creating nonuniform anisotropy.<sup>7–9</sup> By slowing conduction and favoring more heterogeneity, these substrate changes are known to promote reentry mechanisms.

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The hexapeptide compound rotigaptide (formerly called ZP123) acts by selectively increasing gap junctional conductance with no other ion channel effects. It reduces gap junction closing during experimental acidosis and ischemia,

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and prevents intercellular uncoupling without any proarrhythmic effects.<sup>10–15</sup> After acute intravenous administration, rotigaptide accelerates atrial conduction in experimental dog models of AF<sup>16,17</sup>; it prevents AF in isolated atrial myocardial ischemia<sup>17</sup> and in a mitral regurgitation model<sup>16</sup> but not in other AF models. GAP-134 is a new modified dipeptide (available orally) that behaves similarly to rotigaptide by specifically enhancing gap-junction conductance with no effect on ion channels or systemic hemodynamics. The present study investigates the effect of oral GAP-134 on conduction abnormalities and AF vulnerability in a novel model of pacing-induced atrial myopathy.<sup>18</sup>

## Methods

Twenty-four age-, weight-, and gender-matched adult mongrel dogs (University of Guelph, ON, Canada) underwent simultaneous atrioventricular pacing (SAVP) at 220 bpm for 14 days; 12 received active drugs and 12 received no drugs (positive controls). Eight UNPACED dogs, composed of 4 implanted dogs with pacemakers turned off (UNPACED-CTRL) and 4 nonimplanted dogs (UNPACED-GAP-134), served as negative controls.

### Pacemaker Insertion for Simultaneous Atrioventricular Pacing

The SAVP model of AF has been previously described in detail.<sup>19</sup> In brief, 2 bipolar screw-in pacing leads (Tendril SDX, St Jude Medical Inc) were fixed in the right atrial appendage and the right ventricular apex and connected to a pacemaker (Verity ADX, St Jude Medical Inc). One week later, the RA and RV were simultaneously paced (atrioventricular delay, 0 ms) at 220 bpm.

### Echocardiographic Studies

Transoesophageal echocardiography (TEE) was performed using a Sonos 5500 (Philips Ultrasound) ultrasound system to measure atrial and ventricular dimensions before pacing, at baseline, and after 14 days of pacing.

Two-dimensional left atrial fraction area shortening and left ventricular fraction area shortening were calculated as previously described<sup>17,18</sup> and expressed as the change from baseline value. To assess the relation between drug effect and the extent of atrial remodeling, for secondary analysis, dogs were divided into above or below the median change from baseline in left atrial systolic area (LASA).

### Electrophysiological Studies

After 14 days of pacing, the pacemaker was turned off 30 minutes before anesthesia with propofol and isoflurane (IV propofol 2.5 to 3.5 mg/kg, followed by isoflurane 1% to 2%); 3 bipolar epicardial electrodes were sewn on the right and left atrial appendages and posterior wall of the left atrium.

A “clock-face electrode” (16 peripheral unipolar electrodes; all equidistant from each other and from a central bipolar electrode; radius, 7.5 mm) was sutured to the epicardium of the posterior left atrium. Intracardiac electrograms were recorded in bipolar mode filtered at 30 to 300 Hz and in unipolar mode (0.05 to 300 Hz), and stored on a custom acquisition system (AQUI 2, Cartesian Labs). Atrial effective refractory periods (AERPs) and Global interatrial conduction times were measured at each of 3 sites at 400- and 200-ms cycle length (CL) pacing at twice the stimulation threshold after 30 seconds of continuous pacing (400- and 200-ms CL) as previously described.<sup>17,18</sup> Using the clockface electrode, local atrial conduction properties at the posterior wall of the left atrium were expressed as the average conduction velocity (CV; cm/s) and the mean wavelength (calculated as mean atrial conduction velocity × mean AERP). CV was calculated from the center of the electrode to the local unipolar electrogram at each of the 16 peripheral electrodes, averaged to obtain a mean CV for all 16 electrodes. A conduction

anisotropic index (fastest CV divided by the slowest) was calculated to analyze the propagation pattern in this specific area.

### Atrial Fibrillation Induction

Burst attempts of atrial pacing (10 V, 10-ms pulse duration at 10 Hz for 10 seconds) were applied 5 times at each of 3 sites or until a total of 10 minutes (600 seconds) of AF was achieved per site (maximum cumulative time spent in AF; 1800 seconds per dog). AF was defined as an irregular atrial arrhythmia with A–A intervals (time interval between 2 consecutive atrial electrograms) less than 150 ms and lasting more than 1 minute. Inducibility and maintenance of AF were defined as follows:

1. Ability to induce AF: the percentage of burst attempts leading to AF episodes (% of attempts leading to AF).
2. Ability to maintain AF: (a) the percentage of dogs with at least one AF episode lasting more than 10 minutes; (b) the mean number of episodes per dog lasting more than 10 minutes; and (c) the median AF duration per dog (expressed as median and 25th to 75th percentile). AF episodes lasting more than 10 minutes were cardioverted using an external biphasic defibrillator (LIFEPAK12, Medtronic Inc).

### Oral GAP-134 Study

Twelve dogs were randomly assigned to be treated with oral GAP-134 (PACED-GAP-134) at 2.9 mg/kg BID (designed to provide a steady state minimum plasma concentration of >100 nmol/L) starting on the first day of pacing, for 14 days. The 12 other dogs (PACED-CTRL) were given placebo tablets. Investigators were blinded to treatment assignment; capsules were labeled “A” or “B,” and the treatment assignment code was kept at Wyeth Research.

Unpaced dogs were divided into 2 groups: 2.9 mg/kg BID of oral GAP-134 (UNPACED-GAP 134, n=4) for 14 days or no treatment at all (UNPACED-CTRL, n=4).

Animal experiments were performed in accordance with the Canadian Council on Animal Care guidelines.

### Determination of Drug Concentrations

Venous samples were drawn before the open-chest study. Plasma GAP-134 concentration was determined using liquid chromatography with tandem mass spectrometry<sup>20</sup> with a lower limit of quantification of 1 ng/mL.

### Frequency Spectrum Analysis

The longest AF episodes per dog were analyzed after the first minute for 30 seconds. The filtered unipolar electrogram from the posterior left atrium during AF was analyzed using Fast Fourier Transform (FFT) calculated on the digitally filtered waveform over a sliding 2-second window of 2048 points every 1 second for a frequency resolution of 0.48 Hz.<sup>21</sup>

Three variables to describe the AF signals were derived:

1. AF dominant frequency (DF): the frequency corresponding to the highest amplitude peak of the filtered Fourier transform of the electrogram.
2. Organization of AF, defined by the organization index (OI): the harmonics of the DF were determined, and the area under the DF and the first 3 of harmonic peaks were calculated over a 1-Hz window. The total area under the frequency spectrum was calculated from 2 Hz to the fifth harmonic. The ratio of the area under the harmonic peaks to the total area was calculated as the OI.
3. Temporal stability of AF: by the standard deviation of the OI calculated during each episode of AF among 4 peripheral equidistant unipolar electrodes of the clockface electrode.

### Histology and Immunohistochemistry

Transmural tissue sections from RAA were stained with Masson Trichrome for interstitial fibrosis analysis using quantitative area ratios (ratio of trichrome staining to all tissue area). Briefly, slides

**Table 1. General Properties at Open-Chest Study**

	UNPACED-CTRL (n=4)	UNPACED-GAP-134 (n=4)	PACED-CTRL (n=12)	PACED-GAP-134 (n=12)
BW, kg	24.5±2.7	23.6±2.0	26.0±2.3	24.6±3.0
SBP, mm Hg	72.5±6.4	67.4±7.9	63.4±8.3	67.5±12.2
DBP, mm Hg	42.8±6.7	45.8±9.8	42.4±8.5	42.2±10.2
LASA, cm <sup>2</sup>	6.3±1.0 (A)	7.2±1.8 (B)	10.4±1.9	10.6±2.5
LADA, cm <sup>2</sup>	9.9±1.1 (A)	10.2±2.3 (B)	12.9±2.0	13.4±2.1
LAFAS, %	36.6±10.4 (A)	33.9±6.6 (B)	19.7±5.1	21.3±8.0
LVSA, cm <sup>2</sup>	7.1±1.9 (A)	7.8±2.3 (B)	9.5±2.3	8.9±2.0
LVDA, cm <sup>2</sup>	12.4±1.8	13.1±2.0	13.4±3.1	12.8±2.0
LVFAS, %	46.8±11.2 (A)	45.3±8.9 (B)	29.2±5.2	30.5±8.1
GAP-134, nmol/L	<2	423±224	<2	557±239

Intergroup differences by ANOVA. No differences were observed between UNPACED groups and between PACED groups regarding hemodynamic and echocardiographic data, except for GAP-134 blood levels. Data labeled "(A)" were statistically different ( $P<0.05$ ) between UNPACED-CTRL and PACED-CTRL groups; "(B)" corresponds to statistically different data between UNPACED-GAP-134 and PACED-GAP-134.

BW indicates body weight; SBP, systolic blood pressure; DBP, diastolic blood pressure; LASA, left atrial systolic area; LADA, left atrial diastolic area; LAFAS, left atrial fractional area shortening; LVSA, left ventricular systolic area; LVDA, left ventricular diastolic area; LVFAS, left ventricular fractional area shortening; GAP-134, plasma concentration of GAP-134.

were tile imaged at 10× magnification; analysis was conducted using Image J v.1.2.8. Images were segmented using HSB color segmentation. A particle analysis algorithm was run and the ratio of total tissue area to Trichrome stained collagen area calculated.

Immunohistochemistry was used to determine the expression and localization of connexin 43 (Cx43) in the myocardium. A rabbit polyclonal antibody (C6219, Sigma–Aldrich Co) raised against Cx43 and an IgG1 isotype control antibody were evaluated on sections from formalin-fixed canine control (nonpaced), PACED-CTRL and PACED-GAP-134 atrial samples at a concentration of 1.25 µg/mL. The principal detection system consisted of a Vector antirabbit secondary (BA-1000) and a Vector ABC-AP kit (AK-5000) with a Vector Red substrate kit (SK-5100). Tissues were also stained with positive control antibodies (CD31 and vimentin) to ensure that the tissue antigens and accessible for immunohistochemical analysis. Only tissues that were positive for CD31 and vimentin staining were selected. The negative control consisted of performing the entire immunohistochemistry procedure on adjacent sections in the absence of primary antibody. Slides were imaged with a DVC 1310C digital camera coupled to a Nikon microscope.

Expression of Cx43 was determined using RNA isolated from frozen canine control (nonpaced), PACED-CTRL and PACED-GAP-134 atrial samples; Taqman analysis was performed using an ABI Prizm 7900 Taqman instrument, and fold change in mRNA was normalized to the ribosomal RNA molecule 18S.

### Western Blot Detection of Cx43

Tissues from left atrial appendage (≈150 mg) were homogenized in Lysis Buffer and clarified by centrifugation (18 000 *g* for 20 minutes at 4°C). Protein concentration was determined with the BCA Protein Assay Kit (Pierce) using bovine serum albumin as the standard. Samples (20 µg) were mixed with NuPAGE LDS Sample Preparation Buffer (Invitrogen Corp) and 1% β-mercaptoethanol, and heated at 70°C for 10 minutes. Samples were fractionated by SDS gel electrophoresis (NuPAGE 4% to 12% Bis-Tris gels; MES-SDS running buffer), transferred to nitrocellulose membranes (0.2-µm pore size), and treated with blocker buffer (LI-COR Biosciences). Membranes were probed with rabbit anti-Cx43 (Sigma C6219) or mouse antiactin (Sigma A1978), followed by washing and treatment with the appropriate IRDye-labeled secondary antibodies (LI-COR Biosciences). Signals were quantified using the Odyssey infrared imaging system (LI-COR Biosciences).

### Data Analysis

Data are expressed as mean±SD. One-way ANOVAs were performed for plasma concentration. Two-way repeated-measures ANOVAs were conducted for AERP comparisons, CV, conduction times, WL, AF vulnerability criteria, and AF pattern criteria in PACED-CTRL and PACED-GAP-134 groups. When ANOVA revealed significant group effects, *t* tests Bonferroni corrected for multiple comparisons were used to evaluate individual mean differences. A 2-tailed  $P<0.05$  was considered statistically significant.

### Statement of Responsibility

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

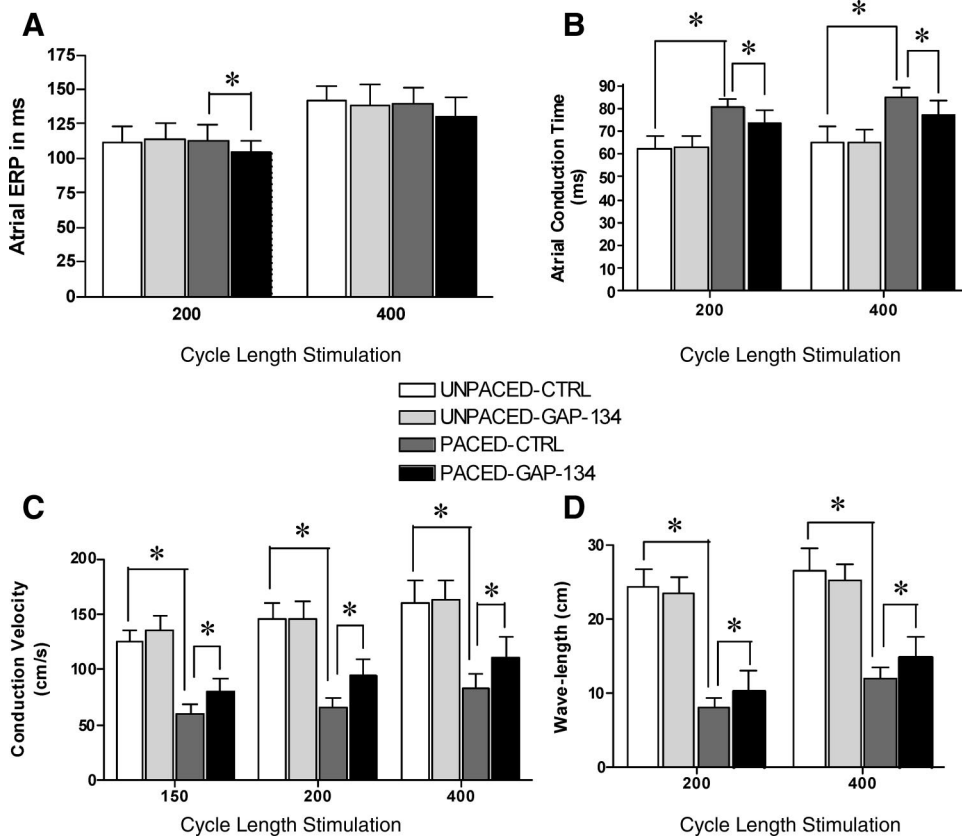
### Results

All animals were alive and in sinus rhythm before the electrophysiological study started on day 14. Neither the hemodynamic parameters nor the echocardiographic parameters were different between the GAP-134 treated versus control UNPACED dogs, and between the GAP-134 treated and placebo treated PACED dogs (Table 1). The median increase in LASA was 100%. The mean plasma concentration of GAP-134 3 hours after the last oral dose was similar between the two treated groups (Table 1).

### Atrial Refractoriness and Atrial Conduction Velocity

In PACED-GAP-134 dogs, the drug caused a nonsignificant shortening in AERP at 400-ms CL stimulation compared with PACED-CTRL dogs (130.4±14.2 versus 140.1±17.0 ms,  $P=NS$ ), and at 200-ms CL stimulation (104.0±8.6 versus 113.0±11.5 ms,  $P<0.05$ ; Figure 1A). GAP-134 caused no changes in AERP in UNPACED dogs. (Figure 1A).

Global mean atrial conduction time was significantly prolonged in both PACED groups compared with UNPACED groups (Figure 1B). Conduction time was significantly shorter in PACED-GAP-134 group (76.7±6.6 ms at 400-ms



**Figure 1.** Mean atrial effective refractory periods (A), mean atrial conduction time (B), mean conduction velocity (C), and wavelength (D) calculated at the posterior wall of the left atrium for all dogs as a function of CL stimulation. \* $P < 0.05$  for intergroup differences by ANOVA.

CL and  $73.2 \pm 5.9$  ms at 200-ms CL) compared with PACED-CTRL group ( $84.9 \pm 4.2$  and  $80.4 \pm 3.4$  ms, respectively).

Local CV was significantly faster in the PACED-GAP-134 group at 400 ( $110 \pm 20$  cm/s), 200 ( $95 \pm 14$  cm/s) and 150-ms CL stimulation ( $80 \pm 12$  cm/s) compared with the PACED-CTRL group ( $83 \pm 13$ ,  $65 \pm 10$ , and  $60 \pm 8$  cm/s, respectively,  $P < 0.05$ ) No differences in conduction time and velocity were observed between UNPACED groups.

The mean WL at the posterior wall of the left atrium (Figure 1D) was significantly shorter in PACED dogs compared with UNPACED dogs. Oral GAP-134 prolonged WL only in the PACED group (PACED-GAP-134 group during pacing at 400-ms CL and at 200-ms CL:  $14.8 \pm 2.9$  and  $10.2 \pm 2.8$  cm versus PACED-CTRL:  $12.0 \pm 1.5$  and  $8.0 \pm 1.4$  cm,  $P < 0.05$ ).

Calculated anisotropic indices were not different between groups (PACED-GAP-134:  $3 \pm 0.9$  versus PACED-CTRL:  $3.44 \pm 1.65$ ).

Figure 2 shows mean atrial WL in the 2 subgroups ( $\Delta\%$  LASA more or less than 100%). When the  $\Delta\%$  in LASA was less than 100%, PACED-GAP-134 dogs had a significant increase in WL when compared with PACED-CTRL (at 200-ms CL:  $12.0 \pm 1.3$  versus  $8.0 \pm 1.4$ ,  $P < 0.05$ , at 400-ms CL:  $13.9 \pm 2.6$  versus  $9.5 \pm 1.9$ ,  $P < 0.05$ ).

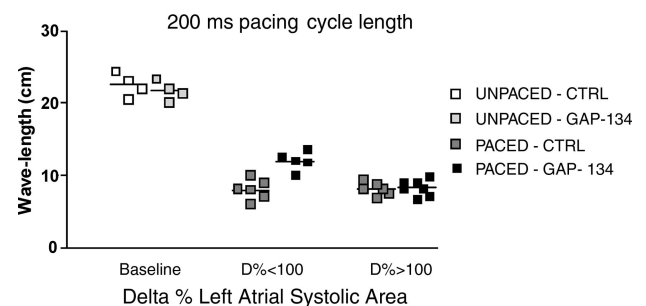
### Vulnerability to Atrial Fibrillation

GAP-134 showed no overall effect in preventing AF inducibility or maintenance (Table 2). Table 3 shows AF vulner-

ability by subgroup; PACED-GAP-134 dogs with less LA dilation (subgroup 4) had a significant reduction in AF inducibility compared with the corresponding CTRL dogs (subgroup 2). The proportion of bursts inducing AF was  $54.0 \pm 24.6\%$  in the CTRL dogs (subgroup 2) and  $15.0 \pm 8.5\%$  in the GAP-134 dogs (subgroup 4), respectively ( $P < 0.05$ ); the mean AF duration was similarly decreased from  $1737 \pm 120$  to  $615 \pm 280$  ( $P < 0.05$ ).

### Histology and Immunohistochemistry

The amount of interstitial fibrosis based on trichrome-positive fibers was scored by a pathologist blinded to the



**Figure 2.** Mean atrial wavelength according to subgroups (more:  $\Delta\%$  LASA  $> 100$  or less:  $\Delta\%$  LASA  $< 100$  than a doubling in LASA between before and after 14 days of pacing) at 200-ms CL stimulation. Baseline values correspond to UNPACED-CTRL and UNPACED-GAP-134 dogs.

**Table 2. AF Vulnerability for All Dogs**

	UNPACED-CTRL (n=4)	UNPACED-GAP-134 (n=4)	PACED-CTRL (n=12)	PACED-GAP-134 (n=12)
% of burst inducing AF	1.0±1.7 [0.5]	1.2±2.3 [0.0]	39.6±26.3† [35]	38.8±23.3‡ [44.6]
% of dogs with AF >10 minutes	0.0 [0.0]	0.0 [0.0]	91.7†	58.‡
No. of episodes >10 minutes	0.0 [0.0]	0.0 [0.0]	2.1±1.0† [2.0]	1.3±1.3‡ [1.0]
Mean AF duration, seconds	35±32 [35]	25±34 [15]	1588±329† [1760]	1256±614‡ [1500]

Data are presented as mean±SD [median]. Intergroup differences were calculated by ANOVA.

†P<0.05, UNPACED-CTRL versus PACED-CTRL.

‡P<0.05, UNPACED-GAP-134 versus PACED-GAP-134.

treatment. Sections were assigned values of either minimal (1) or mild (2) fibrosis. There was no fibrosis more severe than mild. The same score was observed for PACED-CTRL and PACED-GAP-134 treated dogs (data not shown). Using a particle analysis algorithm, no difference in fibrosis was observed. PACED-CTRL left atrial samples had 33.9±2.6% trichrome stained collagen, whereas PACED-GAP-134 left atrium had 29.7±2.4% trichrome stained collagen. Samples showed significant collagen staining within the epicardial and pericardial regions, which likely masked any possible difference in the degree of fibrosis within the interstitium.

PACED-CTRL and PACED-GAP-134 hearts showed similarly strong Cx43 staining of intercalated discs. In normal UNPACED dog hearts, there was also moderate to strong granular cytoplasmic staining (some consistent with Golgi region) and perinuclear staining as well as faint-to-moderate enhancement of the myofibrils and cross-striations. Both PACED-CTRL and PACED-GAP-134 hearts had less staining in these regions. Staining of the lateral cytoplasmic membranes was less evident in the normal hearts than in the PACED-CTRL and PACED-GAP-134 hearts according to a blinding scoring.

Taqman mRNA analysis of Cx43 and Cx40 revealed no difference between PACED-CTRL and PACED-GAP-134 hearts consistent with the immunohistochemistry results. Mean Cx43 mRNA levels normalized to 18S were 1.01±0.29 in PACED-CTRL left atria and 0.92±0.26 in PACED-GAP-134 left atria. Mean Cx40 mRNA levels were 0.74±0.07 in PACED-CTRL and 0.70±0.15 in PACED-GAP-134. No differences in Cx40 or Cx43 mRNA levels were observed in the secondary analysis with dogs separated into groups with greater or less than 100% increase in LASA. Quantitative

Western blot analysis of total Cx43 also failed to show any difference between PACED-CTRL and PACED-GAP-134 treated dogs (data not shown).

**AF Pattern Analysis**

AF pattern outcomes are displayed in Table 4. The DF was higher in PACED-GAP-134 dogs than in PACED-CTRL dogs, suggesting faster conduction or shorter refractoriness during AF. Although the mean OI was unchanged between the two groups, the mean standard deviation of the OI was increased in the PACED-GAP-134 group, reflecting that the AF spatial organization was more variable in this group. Figure 3 displays representative examples of raw electrograms and frequency spectra in a PACED-CTRL (Figure 3A) and PACED-GAP-134 (Figure 3B) dog.

**Discussion**

The main results of this study indicate that, as previously described, pacing the right atrium and ventricle simultaneously induced severe left atrial dilation and a decrease in left atrial WL resulting in AF vulnerability.<sup>19,26,27</sup> The anti-arrhythmic peptide reduced the magnitude of decrease in WL and attenuated AF vulnerability in a subgroup of paced dogs with less left atrial dilation.

**Atrial Refractoriness and Atrial Conduction Velocity**

Oral GAP-134 decreased atrial ERP (at 200 ms pacing CL) only in PACED dogs, but had no such effect in UNPACED dogs. This observation has previously been made after rotigaptide infusion in the mitral regurgitation dog model.<sup>16</sup> Whereas these compounds have no effect on ion channels, an

**Table 3. AF Vulnerability According to Subgroups (More: %Δ LASA >100 or Less: %Δ LASA <100 Than a Doubling in LASA Between Before and After 14 Days of Pacing)**

Subgroup Analysis	Δ%LASA >100 (n=13)		Δ%LASA <100 (n=11)	
	PACED-CTRL Subgroup 1 (n=6)	PACED-GAP-134 Subgroup 3 (n=7)	PACED-CTRL Subgroup 2 (n=6)	PACED-GAP-134 Subgroup 4 (n=5)
% burst inducing AF	35.3±20.6 [35]	55.9±11.7 (A) [55]	54.0±24.6 (B) [63]	15.0±8.5 [17]
% dogs with AF >10 minutes	100	71.4 (A)	100 (B)	40
No. of episodes >10 minutes	1.8±1.2 [2]	1.8±1.3 (A) [2]	2.3±0.8 (B)	0.4±0.6 [0]
Mean AF duration, seconds	1441±415 [1555]	1714±227 (A) [1800]	1737±120 (B) [1800]	615±280 [620]

Data are presented as mean±SD [median]. Data labeled “(A)” are significantly different (P<0.05) between PACED-GAP-134 subgroup 3 (more than a doubling in LASA) and subgroup 4 (less than a doubling in LASA). Data labeled “(B)” are significantly different (P<0.05) between PACED-CTRL and PACED-GAP-134 in the subgroup with less than a doubling in LASA (subgroup 2, PACED-CTRL; subgroup 4, PACED-GAP-134).

**Table 4. AF Pattern Outcomes (Frequency Spectrum Analyses) According to Groups**

	PACED-CTRL (n=12)	PACED-GAP-134 (n=12)	P*
Mean DF, Hz	7.88±0.86	8.92±1.27	<0.001
SD DF	0.76±1.13	1.20±1.21	0.12
Maximum DF, Hz	9.91±3.53	11.89±3.61	0.02
Mean OI	0.56±0.09	0.53±0.09	0.21
SD OI	0.06±0.04	0.09±0.04	0.001

Signals were analyzed from the unipolar electrogram in the posterior left atrium using fast Fourier transform analysis.

increase in tissue excitability may explain this phenomenon. The increase in global and local conduction velocity in PACED-dogs confirmed that this new oral compound is acting similarly to the IV parent compound, rotigaptide.<sup>16,17</sup> Whereas oral GAP-134 partially prevented the decrease in calculated left atrial WL, it had no effect on UNPACED dogs suggesting that this drug did not modify electrophysiological properties in healthy tissues. Drug effects were particularly pronounced in the subgroup of dogs with less left atrial remodeling. Whereas WL values did not return to the normal range of values as measured in UNPACED dogs, the magnitude WL increase (compared with control) may be sufficient to prevent reentry in this model. This phenomenon would be expected to increase the probability that an episode of AF would spontaneously terminate.<sup>22</sup> Ion channel active antiarrhythmic drugs increase atrial WL, by prolonging refractoriness in the atria or widening of the excitable gap during AF,<sup>22</sup>

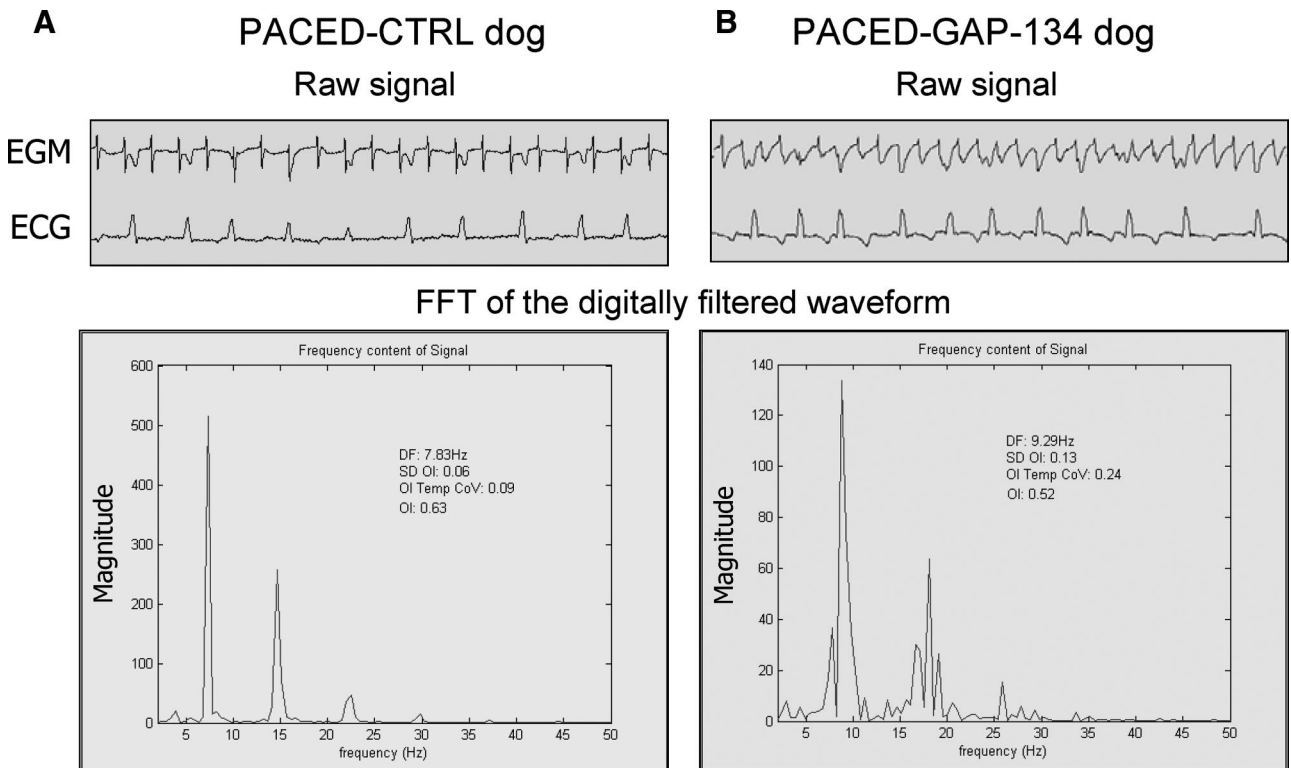
leading to a reduction in the number of functional re-entry circuits and, eventually, failure of reentrant excitation. However, reverse use-dependent effect on refractoriness of class III drugs limit their efficacy against AF.<sup>23</sup>

In our study, GAP-134 increased the left atrial WL by a novel mechanism of action, by increasing the CV rather than prolonging refractoriness. Increasing the size of the pathlength that an atrial wavefront travels in 1 revolution may result in fewer circulating wavelets in the available tissue mass.

**AF Vulnerability**

We observed no overall effect of oral GAP-134 on AF vulnerability in the primary analysis. In a secondary analysis, we observed that PACED-GAP-134 dogs with less than a doubling in LASA had less AF inducibility and maintenance than the corresponding controls. This observation suggests that dogs with marked structural remodeling may have such profound fibrosis or disruption of cell-to-cell communication that the gap junction conductance-enhancing drug may not affect AF inducibility or maintenance.

The observation that the magnitude of the drug effect on WL was more pronounced in the subgroup 4 (less left atrial remodeling) also supports this speculation. Studies with the larger hexapeptide, rotigaptide, have demonstrated that improvements in conduction within the fibrillating atrium are not effective at reducing AF duration or inducibility when the substrate is severely fibrotic in advanced heart failure.<sup>16,17</sup> The tendency of atrial or ventricular fibrillation to terminate spontaneously in finite-sized tissue is known as the critical



**Figure 3.** Representative examples of raw electrograms (recorded at the posterior wall of the left atrium), surface ECG (lead II), and corresponding magnitude spectrums (fast Fourier transform of the digitally filtered waveform) of a PACED-CTRL (A) and PACED-GAP-134 (B) dogs.

mass hypothesis.<sup>24</sup> We speculate that GAP-134 had no beneficial effects on markedly enlarged left atria in prolonging WL and preventing AF because the atrial critical mass “threshold” above which AF is less likely to terminate was reached in this group.

Importantly, we observed similar AF vulnerability in the PACED control groups with greater and lesser atrial size change. This suggests that AF production in this dog model is valid for drug testing because it allows for reliable sustained AF production.<sup>18</sup>

### Atrial Fibrillation Pattern

The AF frequency was higher and the organization was less in PACED-GAP-134 dogs compared with PACED-CTRL dogs, suggesting that on average arrhythmias were more “chaotic.” Because we only analyzed left atrial signals, we cannot assess organization in other areas of the atria. We speculate that GAP-134 may be responsible for changing the AF pattern in a way that may increase the probability that wavefronts would collide and that an episode of AF would spontaneously terminate. Computer simulations<sup>24</sup> have shown that dynamical instability promotes wave breaks, maintaining fibrillation, but it also causes the waves to extinguish, facilitating spontaneous termination of fibrillation. The latter effect seems to predominate as dynamical instability increases, so that fibrillation is more likely to self-terminate in a finite-sized tissue. To better understand mechanisms by which GAP-134 may help to terminate AF episodes, more detailed electrophysiological mapping would be needed.

### Connexin Function and Atrial Fibrillation

Cx43 channels are regulated by a number of signal transduction cascades (kinases and phosphatases) that may be involved in remodeling<sup>8</sup> and which alter the phosphorylation of the protein. Such remodeling might also be the result of fibrosis that disrupts normal gap junction connections.<sup>9</sup> Modifications of atrial gap junction gating, expression, and distribution may be potential new targets in the treatment of atrial reentry arrhythmias.<sup>10</sup> Rotigaptide has been shown to increase atrial CV in various AF dog models, but to suppress AF only for the mitral regurgitation<sup>16</sup> and the acute ischemia<sup>17</sup> substrates. However, none of these studies has proven that the drug may modify the overall level of Cx43 expression or change the phosphorylation status of the protein. To date an increase in Cx43 expression has only been shown in culture after 24-hour incubation.<sup>25</sup> In the present study, we did not observe any changes in transcription of Cx43 or Cx40 or spatial distribution of immunolabeled Cx43 in the atria after 14 days of oral dosing with GAP-134.

Cx43 dephosphorylation in response to low-flow ischemia may be prevented by rotigaptide in perfused rat and guinea pig hearts.<sup>26</sup>

Our study is consistent with previous results and confirms that the enhancement of gap junctional communication is likely to be elicited by an indirect route because rotigaptide did not modify Cx43 colocalization in intercalated discs or Cx43 mRNA levels.

### Potential Limitations

Although the animal model used in this study induces clinically relevant atrial remodeling, it remains a surrogate for human AF. This model can only help us to better understand the possible mechanisms of action of this new oral compound. Unlike spontaneous human AF, but similar to other animal models, burst pacing is required to induce AF.

The plasma concentrations of GAP-134 varied, but plasma levels in all groups were well within the maximally effective concentration range (10 to 300 nmol/L) defined in previous studies.<sup>27</sup>

The apparent beneficial effect of GAP-134 on AF in dogs with less atrial remodeling was only observed in a secondary post hoc analysis, and prospective confirmation of this hypothesis would be useful.

Another potential limitation is that the study was done in animals anesthetized with isoflurane, a compound known to be a partial gap junction uncoupler.<sup>28</sup> However, the fact that isoflurane was kept constant during the course of the study makes it unlikely that the anesthesia played a significant role in the results.

### Conclusions

Gap junction modulation with chronic oral GAP-134 improved atrial conduction in a model of atrial myopathy. This new antiarrhythmic peptide appeared to prevent AF inducibility and maintenance in dogs with less change in left atrial size after 14 days of SAVP. Our results suggest that this novel compound may have a potential effect when administered before the atrial substrate for AF is severely altered.

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### Disclosures

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### CLINICAL PERSPECTIVE

Abnormal cell-to-cell communication caused by connexin dysfunction may constitute a mechanism for atrial fibrillation. In an animal model of atrial fibrillation caused by rapid simultaneous atrioventricular pacing, we tested the effect of an orally active gap junction-enhancing antiarrhythmic peptide (GAP-134) on atrial conduction velocity, refractoriness, and inducibility of atrial fibrillation. GAP-134 increased the velocity of atrial conduction. Although vulnerability to induced atrial fibrillation was not changed in the entire sample, in a subset of dogs with less than a doubling of left atrial surface area, GAP-134 significantly reduced the probability of atrial fibrillation induction from 100% to 40% and reduced mean atrial fibrillation duration from  $1737 \pm 120$  to  $615 \pm 280$  seconds ( $P < .05$ ). Enhancing gap junction conduction in mechanically remodeled atria may reduce vulnerability to atrial fibrillation, particularly if the remodeling is less pronounced.