The Gap Junction Modifier, GAP-134 [(2S,4R)-1-(2-Aminoacetyl)-4-benzamido-pyrrolidine-2-carboxylic Acid], Improves Conduction and Reduces Atrial Fibrillation/Flutter in the Canine Sterile Pericarditis Model

Eric I. Rossman, Kun Liu, Gwen A. Morgan, Robert E. Swillo, Julie A. Krueger, Stephen J. Gardell, John Butera, Matthew Gruver, Joel Kantrowitz, Hal S. Feldman, Jørgen S. Petersen, Ketil Haugan, and James K. Hennan

Cardiovascular and Metabolic Disease (E.I.R., K.L., G.A.M., R.E.S., J.A.K., S.J.G., J.K.H.), Chemical and Screening Sciences (J.B.), and Drug Safety and Metabolism (M.G., J.K., H.S.F.), Wyeth Research, Collegeville, Pennsylvania; and Zealand Pharma A/S, Glostrup, Denmark (J.S.P., K.H.)

Received December 19, 2008; accepted February 20, 2009

ABSTRACT
Gap junction uncoupling can alter conduction pathways and promote cardiac re-entry mechanisms that potentiate many supraventricular arrhythmias, such as atrial fibrillation (AF) and atrial flutter (AFL). Our objective was to determine whether GAP-134 [(2S,4R)-1-(2-aminoacetyl)-4-benzamido-pyrrolidine-2-carboxylic acid], a small dipeptide gap junction modifier, can improve conduction and ultimately prevent AF/AFL. In rat atrial strips subjected to metabolic stress, GAP-134 prevented significantly conduction velocity slowing at 10 nM compared with vehicle (p < 0.01). In the canine sterile pericarditis model, conduction time (CT; n = 5), atrial effective refractory period (AERP; n = 3), and AF/AFL duration/inducibility (n = 16) were measured 2 to 3 days postoperatively in conscious dogs. CT was significantly faster after GAP-134 infusion (average plasma concentration, 250 nM) at cycle lengths of 300 ms (66.2 ± 1.0 versus 62.0 ± 1.0 ms; p < 0.001) and 200 ms (64.4 ± 0.9 versus 61.0 ± 1.3 ms; p < 0.001). No significant changes in AERP were noted after GAP-134 infusion. The mean number of AF/AFL inductions per animal was significantly decreased after GAP-134 infusion (2.7 ± 0.6 versus 1.6 ± 0.8; p < 0.01), with total AF/AFL burden being decreased from 12,280 to 6063 s. Western blot experiments showed no change in connexin 43 expression. At concentrations exceeding those described in the AF/AFL experiments, GAP-134 had no effect on heart rate, blood pressure, or any electrocardiogram parameters. In conclusion, GAP-134 shows consistent efficacy on measures of conduction and AF/AFL inducibility in the canine sterile pericarditis model. These findings, along with its oral bioavailability, underscore its potential antiarrhythmic efficacy.

In the myocardium, propagation of the action potential between adjacent cardiac myocytes occurs by ion transfer through transmembrane channels, called gap junctions. These membrane-spanning channels, localized at the intercalated disc, are composed of two connexon hexamers, one from each adjacent cell, coupled together. Each connexon is formed by the oligomerization of six protein molecules called connexins (Cxs). During electrical stimulation, gap junction coupling is a critical determinant of conduction velocity. In cardiac diseases, such as heart failure and ischemic heart disease, gap junctional uncoupling, because of changes in Cx expression and/or phosphorylation, is well documented (Peters et al., 1993; Matsushita et al., 1999; Dupont et al., 2001b; Akar et al., 2004; Severs et al., 2004; Ai and Pogwizd, 2005; Saffitz et al., 2007). Current theories suggest that these alterations in cell-to-cell coupling can alter conduction pathways and promote cardiac re-entry mechanisms that potentiate many supraventricular arrhythmias, such as atrial fibrillation (AF) and atrial flutter (AFL) (van der Velden and Jongsm, 2002; Ehrlich et al., 2007; Nattel et al., 2007).

AF is the most common sustained cardiac arrhythmia in the adult population, with 25 to 35% of these patients also...
showing evidence of AFL (Blomström-Lundqvist et al., 2003; Fuster et al., 2006). In addition, AF is the most frequent complication after cardiac surgery (Echahidi et al., 2008). This postoperative AF can be life threatening and is associated with a significant increase in morbidity and mortality, particularly in the elderly population. Patients suffering from postoperative AF have an increased risk of stroke, hemodynamic compromise, and ventricular dysrhythmias. Moreover, postoperative AF was estimated to be 4.9 days, and with approximately 700,000 open heart surgeries per year in the United States, the extra cost has been estimated to be over $2 billion/year (Aranki et al., 1996; Echahidi et al., 2008). It is unfortunate that even with this enormous clinical impact and financial burden, there is no approved preventive treatment for postoperative AF.

In this regard, we recently identified the first, orally available, gap junction modifier, GAP-134, which is a dipetide chemically derived from the class of antiarrhythmic peptides such as rotigaptide (also called ZP123) (Butera et al., 2009). GAP-134 dose-dependently reduced calcine dye uptake in C6 cells overexpressing Cx43 and prolonged time to cardiac conduction block in a mouse model of calcium overload (Butera et al., 2009). In Butera et al. (2009), the efficacy and potency of GAP-134 were directly compared with rotigaptide and found to be similar. In the present study, we add additional knowledge regarding the mechanism of action of GAP-134 by investigating its action on conduction velocity before and after metabolic stress, using rat atrial muscle strips. It is typical that under ischemic conditions, gap junction uncoupling occurs, leading to a reduced conduction velocity. However, administration of GAP-134 prevented any such conduction slowing, emphasizing its ability to maintain cell-to-cell coupling. In whole-animal studies, we tested the hypothesis that increased gap junctional conductance after GAP-134 administration would prevent conduction abnormalities and the promotion of cardiac re-entry mechanisms associated with AF and AFL. We specifically measured the ability of GAP-134 to limit the inducibility and duration of pacing-induced AF/AFL episodes during canine sterile pericarditis. We also provide a detailed cardiovascular safety assessment of GAP-134 in telemetry-instrumented conscious dogs, which underscores the potential utility of GAP-134 as a prophylactic therapy for this common and troublesome postoperative arrhythmia.

Materials and Methods

All investigations were conducted in accordance with the Guide for Care and Use of Laboratory Animals (NIH no. 86-23).

Materials. Wyeth Research (Collegeville, PA) manufactured GAP-134 (molecular mass of 291.3 g/mol), and the chemical structure is presented in Fig. 1. All other reagents were purchased from commercial suppliers.

Fig. 1. Chemical structure of GAP-134.

Rat Atrial Strip Study. Male Sprague-Dawley rats weighing 350 to 450 g were sacrificed by CO2 asphyxiation. The left atrium was carefully dissected, and tissue samples of approximately 2 × 6 mm were taken from the free wall and placed in a tissue chamber perfused throughout the study with 37°C oxygenated Tyrode's buffer containing 136.0 mM NaCl, 4.0 mM KCl, 0.8 mM MgCl2, 1.8 mM CaCl2, 5.0 mM HEPES, 5.0 mM MES, and 6.0 mM glucose, pH 7.3. The atrial tissue was fixed to two hooks in the chamber by small bulldog clamps. A bipolar stimulation electrode (Teflon-coated stainless steel) one end of the tissue. Stimulation was performed at a cycle length of 1000 ms using rectangular pulses at double threshold (0.2 ms pulse duration). Two separate microelectrodes of pure iridium (World Precision Instruments, Inc., Sarasota, FL; tip impedance, 3.5–4.0 mol wt.) were placed on a line along the long axis of the preparation for recording of atrial conduction velocity (CV). The distances from the stimulating electrode to the first and second microelectrode was 1.5 to 2.0 and 3.0 to 4.0 mm, respectively. After a 30-min equilibration period, pacing was initiated, and the atria were superfused during four experimental periods as follows: baseline, 20 min with oxygenated Tyrode’s buffer; treatment, 20 min with oxygenated Tyrode’s buffer with GAP-134 (10 nM) or rotigaptide (10 nM) or saline vehicle; metabolic stress, 40 min with nonoxygenated, glucose-free Tyrode’s buffer with GAP-134 (10 nM) or rotigaptide (10 nM) or saline vehicle; and recovery, 20 min with oxygenated Tyrode’s buffer with GAP-134 (10 nM) or rotigaptide (10 nM) or saline vehicle.

Creation of the Canine Sterile Pericarditis Model. Male mongrels (n = 35) weighing 20 to 25 kg were anesthetized with sodium pentobarbital (30 mg/kg i.v.). The animals were intubated and ventilated with room air using a Harvard respirator (Harvard Apparatus Inc., Holliston, MA), adjusted to deliver 400 ml/breath at a frequency of 12 breaths/min. The sterile pericarditis model was created as described previously (Pagé et al., 1986). At the time of surgery, pairs of platinum needle electrodes (Astro-Med, West Warwick, RI) were placed on the right atrial appendage, the Bachmann’s bundle, and the posteroinferior left atrium (PLA) in all dogs. Intra- and postoperative care included the administration of cefazolin (20 mg/kg) b.i.d. for 2 days and buprenorphine (0.01 mg/kg) b.i.d. for 1.5 days.

Canine Electrophysiologic and Induction Studies. On postoperative days 2 and/or 3, atrial electrophysiologic and induction studies were performed in conscious dogs. Conduction time (CT) was determined at cycle lengths of 300 and 200 ms, using twice-threshold stimuli (pulse duration, 1.8 ms). CT was measured from the Bachmann’s bundle to the PLA, using stimuli from the right atrial appendage electrode. After CT measurements, induction of AF/AFL was attempted using a burst pacing protocol. Standardized rapid atrial pacing, using twice-threshold stimuli (pulse duration, 1.8 ms; 5-s train duration), was performed on all dogs at each electrode site starting at 500 bpm and increasing at increments of 20 bpm until 800 bpm (48 total inductions). Only periods of AF/AFL lasting greater than 1 min were considered sustained AF/AFL and were used for analysis. Sustained AF/AFL (>15 min) that did not spontaneously terminate was cardioverted using standard pacing techniques. Upon completion of baseline AF/AFL induction, GAP-134 or vehicle (saline) was administered intravenously with a bolus infusion of 25.0 µg/kg followed by a maintenance infusion of 19.0 µg/kg/h. After 20 min, CT was remeasured, and AF/AFL was rebounded using the same standardized techniques as described previously (48 total inductions). Plasma samples were taken at the time of the study to determine concentration of GAP-134. Before euthanasia, dogs were anesthetized with sodium pentobarbital (30 mg/kg i.v.), and left atrial tissue samples were dissected free and immediately snap-frozen in liquid nitrogen for Western blot analysis.

In a separate subset of dogs, on postoperative day 3, the atrial effective refractory period (AERP) was measured, at the PLA site, using a premature stimulus protocol before and after GAP-134 or vehicle treatment. After a train of stimuli (S1) at cycle lengths

Fig. 1. Chemical structure of GAP-134.
of 300 and 200 ms, a single premature stimulus (S₂) was introduced. The coupling interval between the last S₁ and the test stimulus (S₂) was progressively shortened in 5-ms steps after every train of stimuli. The longest S₁-S₂ interval for a stimulus that failed to produce an atrial response was defined as the AERP.

Cardiovascular Safety Profile. The potential effects of GAP-134 on the rapidly activating delayed rectifier cardiac potassium ion current (I₉⁄₆) were examined in the in vitro hERG potassium ion channel assay. Human embryonic kidney 293 cells that were stably transfected with hERG cDNA were studied using patch-clamp electrophysiology techniques. Cells were transferred from the incubator to the recording chamber and superfused with vehicle solution, HEPEs-buffered physiologic saline and 0.1% dimethyl sulfoxide. The recording chamber and bathing solution were maintained at a temperature range of 35 ± 2°C. A commercial patch-clamp amplifier was used for whole-cell recordings. Cells stably expressing hERG were held at a resting membrane potential of ~80 mV. Onset and steady-state block of hERG current due to GAP-134 (at concentrations of 10, 100, and 1000 μM), positive control article (terfenadine, a drug known to block hERG current), or vehicle were measured using a stimulus pulse pattern repeated at 5-s intervals. Peak test pulse current was measured during the test ramp. A steady state was maintained for at least 20 s before applying test article, positive control, or vehicle control. Peak current was monitored during repetitive stimulation until a new steady state was achieved. The percentage inhibition in each recorded cell was calculated by normalizing peak current at steady state during test article, positive control, or vehicle control application to the peak current at steady state before application.

To further understand the cardiovascular safety, GAP-134 was administered to male and female dogs (five per gender) as a single intravenous bolus at suprapharmacological doses of 0 (saline control), 5, 15, or 50 mg/kg according to a Latin square crossover dosing paradigm. Parameters collected using radiotelemetry consisted of arterial blood pressures (systolic, diastolic, and mean), heart rate, and lead II ECG. The telemetry data were collected for 30-s periods every 5 min, for 24 h before and 24 h after dosing with saline control or GAP-134. Effects on heart rate and arterial blood pressure were evaluated at 15-min time points in all animals at all dosages for 24 h before and 24 h after each dosage. Effects on the ECG were evaluated at selected time points using data from animals given saline control and 50 mg/kg GAP-134. ECG measurements were performed at 0.5, 3, 9, and 23 h after dosing to ensure characterization of potential GAP-134 effects across a range of plasma test article concentrations based on previous pharmacokinetic studies (data not shown).

Determination of Plasma Concentration. A bioanalytical liquid chromatography/tandem mass spectrometry method using a protein precipitation extraction procedure was used to determine GAP-134 concentrations in dog plasma. A stable-labeled compound was used as the internal standard. Extracted samples were chromatographed on a Phenomenex Synergi Hydro-RP column (50 × 2.0 mm, 4 μm; Phenomenex, Torrance, CA) using high-performance liquid chromatography gradient elution. Tandem mass spectrometry analysis was performed using electrospray ionization in positive ion mode. Concentrations of GAP-134 in dog plasma were determined by a weighted (1/concentration²) least-squares linear regression method. Based on a 0.1-ml sample volume, the lower limit of quantitation was 2.00 ng/ml, and the concentration range was 2.00 to 2000 ng/ml.

Western Blot Analysis. Frozen canine atrial heart specimens (~150 mg) were homogenized in lysis buffer (25 mM Tris, pH 7.6, 0.1M NaCl, 5 mM EDTA, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 1× protease inhibitors, 1× phosphatase inhibitors). The homogenate was clarified by centrifugation (18,000 g; MES-SDS running buffer), transferred to nitrocellulose membranes (0.2-μm pore size), and treated with blocker buffer (LI-COR Biosciences, Lincoln, NE). Membranes were probed with rabbit anti-connexin43 (C8219, Sigma-Aldrich, St. Louis, MO) or mouse anti-actin (A1978, Sigma-Aldrich), followed by washing and treatment with the appropriate IRDye-labeled secondary antibodies (LI-COR Biosciences). Signals were detected and quantified using the Odyssey infrared imaging system (LI-COR Biosciences).

Statistical Analysis. Results are presented as mean ± S.E.M. for CV, conduction time, and induction parameters. In the rat atrial strip experiment, 5-min interval CV data were analyzed by a two-way ANOVA model with repeated measure. In the canine AF studies, conduction time was analyzed by standard Student's paired t tests. Duration and induction data were analyzed by two-way ANOVA models with repeated measures using post hoc least significant difference tests. Induction data were given a square-root transformation before the model fitting to make the frequency data close to normal distribution. In the safety pharmacological studies, a repeated measures ANOVA was used to compare mean response after administration of GAP-134 to mean response after saline administration. The ANOVA model used for heart rate data analysis included random effect to account for animal-to-animal differences. For heart rate-corrected QT comparisons, a study-specific linear regression model was fit to the combined predose data for saline control and compound dosages. For all statistical analysis, p < 0.05 was considered significant.

Results

Effects of GAP-134 on Conduction and AERP. In rat atrial strips, CV was measured during four experimental periods while being superfused with saline vehicle, GAP-134, or rotigaptide (Fig. 2). During the baseline and treatment periods (0–40 min) without metabolic stress, CV was stable, and there were no differences between groups. At the end of the metabolic stress period (80 min), in the saline vehicle-treated group, atrial CV significantly decreased by −24.5 ± 2.8% (p < 0.0001) compared with baseline values. No signif-
significant changes, at the end of the metabolic stress period, were noted for GAP-134- and rotigaptide-treated groups compared with baseline values. However, upon completion of the metabolic stress period (80 min), atrial CV for the GAP-134-treated group was significantly higher compared with the saline vehicle group (GAP-134 versus vehicle \( p < 0.01 \)). A similar effect was observed with 10 nM rotigaptide, which has been shown previously to inhibit stress-induced conduction slowing (rotigaptide versus vehicle \( p < 0.01 \)) (Haugan et al., 2005).

In the canine sterile pericarditis model, CT was determined at cycle lengths of 300 and 200 ms, before and after GAP-134 administration (average plasma concentration, 73.1 ng/ml; 250 nM). As shown in Fig. 3, GAP-134 significantly reduced CT at cycle lengths of 300 ms (66.2 ± 1.0 versus 62.0 ± 1.0 ms; \( p < 0.001 \)) and 200 ms (64.4 ± 0.9 versus 61.0 ± 1.3 ms; \( p < 0.001 \)). In a subset of dogs (\( n = 3 \)), AERP was measured, before and after GAP-134 administration, at cycle lengths of 300 and 200 ms. GAP-134 did not significantly alter the AERP at either cycle length (300 ms, 106.7 ± 4.4 versus 105.0 ± 6.3 ms; 200 ms, 109.2 ± 4.2 versus 113.3 ± 1.7 ms). Although the small sample size used to measure AERP in this study is a limitation, a recent study by Dr. Paul Dorian thoroughly explored the effect of GAP-134 on AERP, and other electrophysiological parameters, in a canine heart failure model of atrial fibrillation (Laurent et al., 2009). In this study, a very small reduction in AERP was observed with GAP-134 at only one cycle length in combination with a large increase in conduction velocity.

**Effects of GAP-134 on AF/AFL Maintenance and Inducibility.** Creation of the sterile pericarditis model in 32 dogs yielded a success rate of only 50%, as defined by the ability to induce sustained AF/AFL (>1 min) after burst pacing. In those animals that were inducible (\( n = 16 \)), the ability to maintain sustained AF/AFL was measured before and after GAP-134 (\( n = 9 \); \( n = 5 \) on postoperative day 2; \( n = 4 \) on postoperative day 3) or vehicle (\( n = 7 \); \( n = 5 \) on postoperative day 2; \( n = 2 \) on postoperative day 3) administration. After GAP-134 treatment, the mean AF/AFL duration per induction (Fig. 4A) was significantly reduced compared with baseline from 603 ± 119 to 254 ± 112 s (\( p < 0.01 \)). No differences were noted in vehicle-treated animals compared with baseline measurements (744 ± 107 versus 608 ± 160 s). Total AF/AFL duration per animal (Fig. 4B) after GAP-134 infusion was also significantly reduced compared with baseline (1364 ± 419 versus 674 ± 452 s; \( p < 0.01 \)), whereas vehicle treatments again yielded no differences (1640 ± 544 versus 1438 ± 587 s). Total AF/AFL burden, a summation of all the AF/AFL durations for each group throughout the entire study, was reduced by 51% from 12,280 s (8434-s AF; 3846-s AFL) to 6063 s (5817-s AF; 246-s AFL) in the GAP-134-treated group compared with baseline, whereas only 12% from 11,478 s (8715-s AF; 2763-s AFL) to 10,064 s (6217-s AF; 3847-s AFL) in the vehicle-treated group compared with baseline induction (Fig. 5).

In those animals that were inducible, the mean number of AF/AFL inductions per animal (Fig. 6) was measured before and after GAP-134 or vehicle administration. In GAP-134-treated animals, the mean number of AF/AFL inductions were significantly reduced from 2.7 ± 0.6 episodes before treatment to 1.6 ± 0.8 episodes after treatment (\( p < 0.01 \)). In these animals, the total number of inductions decreased from 24 episodes (of which 12 needed to be cardioverted for sustained runs of AF/AFL lasting >15 min) to 14 episodes (four cardioversions needed) after GAP-134 treatment. No significant reductions were noted in the vehicle-treated animals; however, the mean number of AF/AFL inductions did decrease slightly from 2.3 ± 0.7 to 1.7 ± 0.7 episodes, with the total number of inductions decreasing from 16 (12 cardioversions needed) to 12 episodes (11 cardioversions needed).

**Western Blot Analysis.** Quantitative Western blot analysis of total Cx43, in left atrial samples, did not show any difference between GAP-134- or vehicle-treated dogs (data not shown).

**Cardiovascular Safety Profile of GAP-134.** GAP-134 did not inhibit the hERG potassium ion current in patch-clamp experiments. Percent inhibition expressed as mean ± S.D. was 0.2 ± 0.4% (\( n = 3 \)) at 10 μM, 0.5 ± 0.1% (\( n = 3 \)) at 100 μM, and 4.0 ± 0.4% (\( n = 3 \)) at 1000 μM. Under identical experimental conditions, the vehicle (HEPES-buffered physiologic saline and 0.1% dimethyl sulfoxide), used as the negative control, resulted in 0.6 ± 0.4% (\( n = 3 \)) inhibition of the hERG potassium ion current. Terfenadine, the positive control, resulted in 0.6 ± 0.4% (\( n = 3 \)) inhibition of the hERG potassium ion current. Terfenadine, the positive control, resulted in 0.6 ± 0.4% (\( n = 3 \)) inhibition of the hERG potassium ion current at a concentration of 60 nM. Because the maximal GAP-134 inhibition of the hERG potassium ion current observed was less than 50%, the IC\textsubscript{50} for the inhibitory effects of GAP-134 on hERG potassium ion current is >1000 μM (291 μg/ml).

Single intravenous (bolus) dosages of 5, 15, or 50 mg/kg administered to male and female dogs did not produce any effects on heart rate, blood pressure, or any ECG parameter (Table 1). There was no evidence of QTc prolongation, abnormal morphologic waveform changes, or abnormal atrial or ventricular arrhythmias in any of the ECGs examined at 0 or 50 mg/kg GAP-134. There were no mortality and no clinical observations associated with this study. After a single intravenous bolus dosage of 50 mg/kg in male and female dogs, the peak blood plasma concentration ([C\textsubscript{peak}] = 190 μg/ml) was approximately 2603 times greater than the efficacious plasma concentration in the canine sterile pericarditis model.
Discussion

The present study is the first demonstration that a gap junction modifier, with capabilities to increase CV, can limit the inducibility and duration of postoperative AF/AFL. In the canine sterile pericarditis model of postoperative AF/AFL, GAP-134 increases CV and significantly reduces AF/AFL duration and inducibility, without affecting the AERP. Cardiovascular safety studies show that GAP-134 does not inhibit the hERG potassium ion current and has no effect on heart rate, arterial blood pressure, or any ECG parameter. Together, these findings underscore the possibility that GAP-134 may be an effective and safe antiarrhythmic compound. Moreover, these data support the notion that enhancing gap junction communication, and thereby improving conduction, can potentially lead to the preventative use of GAP-134 as a treatment for postoperative AF.

Abnormal conduction, resulting from alterations in Cx expression and distribution, is quite evident and plays a pathologic role in AF. Studies on atrial tissue, looking at Cx changes, from patients suffering from AF have reported varying results, with some showing a net increase in Cx40 and/or Cx43 (Dupont et al., 2001a; Polontchouk et al., 2001; Wetzel et al., 2005), whereas others describe a decrease in either Cx40 and/or Cx43 (Kostin et al., 2002; Nao et al., 2003; Wilhelm et al., 2006). Within these studies, investigators also have revealed a heterogenous distribution of Cx40 and/or Cx43 from the intercalated disc to the lateral borders of the myocytes (Polontchouk et al., 2001; Kostin et al., 2002). In addition to these changes, phosphorylation changes have also been identified. A study by Nao et al. (2003) compared atrial tissue from patients with AF to those in normal sinus rhythm and showed a marked increase in the serine-phosphorylated levels of Cx40. Independent of the specific change, these alterations can lead to heterogeneity in the anisotropy of conduction, promotion of re-entrant pathways, and ultimately AF/AFL.

Postoperative AF occurs in approximately 30% of patients after isolated coronary artery bypass grafting surgery, 40% after valve replacements, and up to 50% for combined procedures (Echahidi et al., 2008). Being that the incidence of postoperative AF is strongly age-dependent, these figures are
expected to rise because the population undergoing cardiac surgery is getting older. Although the mechanisms underlying the abnormal conduction are multifactorial, Cx alterations are again present and quite variable. To be specific, Dupont et al. (2001a) showed no changes in Cx43 and Cx45 mRNA and protein levels, whereas CX 40 levels were increased in atria from postoperative AF patients. On the contrary, Wilhelm et al. (2006) found that the Cx40/Cx45 ratio was significantly reduced in atria from postoperative AF patients compared with pre- and postoperative patients. Both studies reported heterogenous distribution of Cx40 in atria from postoperative AF patients. It is unfortunate that interpatient variability among postoperative AF patients can make these findings difficult to interpret, thus highlighting a need for reliable animal models. The canine sterile pericardium model has been a well established representation of human postoperative AF/AFL for many years. A recent study by Ryu et al. (2007), looking at gap junctional changes in the canine sterile pericarditis model, showed differences in the overall expression and transmural distribution of Cx40 and Cx43, similar to the human disease. In this study, the authors concluded that the aforementioned Cx changes are associated with abnormal atrial conduction and can lead to increased AF/AFL vulnerability. In our study, the ability of GAP-134 to increase CV while reducing AF/AFL duration and inducibility strongly supports these findings.

Some investigators have linked the aforementioned electrical abnormalities and increased AF/AFL inducibility to the acute phase of inflammation after cardiac surgery (Gaudino et al., 2003; Kumagai et al., 2004; Ishii et al., 2005; Tselentakis et al., 2006; Goldstein et al., 2008). In the canine sterile pericarditis model of postoperative AF/AFL, Tselentakis et al. (2006) found that anti-inflammatory drugs, ibuprofen and methylprednisolone, when applied before surgery, reduced the incidence of sustained AF. In the Tselentakis study, researchers explored the link between inflammation and conduction by studying electrical properties of canine atrial appendages subjected to the inflammatory mediator, arachidonic acid. The authors were able to demonstrate that acute inflammation, mimicked by arachidonic acid, reduced CV and produced anisotropy. In addition to promoting cardiac re-entry and ultimately AF, these findings establish the role of conduction abnormalities as the underlying pathologic mechanism within inflammation in the setting of postoperative AF. Although anti-inflammatory treatment for the prevention of postoperative AF looks promising, the results of a recent cohort study from the AF Suppression Trials I, II, and III highlighted some potential safety concerns (Ruffin et al., 2008). To be specific, nonsteroidal anti-inflammatory drugs, although efficacious in reducing postoperative AF, also increased the incidence of stroke and myocardial infarction. Again, the ability of GAP-134 to treat the underlying conduction abnormalities, possibly associated with inflammation, without any apparent safety concerns truly differentiates and illustrates its antiarrhythmic potential in treating postoperative AF.

Most patients who present with AF after cardiac surgery adhere to conventional treatment strategies, including prevention of thromboembolic events, control of the ventricular rate response, and restoring/maintenance of sinus rhythm. Nevertheless, for these patients undergoing surgery, there is currently no standard of care for the prophylactic treatment of postoperative AF. The most common preoperative interventions include administration of amiodarone, β-adrenergic blockers, sotalol, or atrial pacing (Crystal et al., 2004; DiDon menico and Massad, 2005). Although these interventions have been shown to reduce the incidence of postoperative AF, it is still controversial whether or not these treatments translate into stroke reduction, decreased perioperative complications, and cost benefits such as reducing hospital length of stay. Therefore, one might suggest that the adverse side effects of these interventions might outweigh the benefits of reducing postoperative AF, thus opening the door for safer, effective compounds, such as GAP-134.

In conclusion, our results demonstrate that increased gap junctional conductance after GAP-134 administration can prevent conduction abnormalities leading to maintenance of sinus rhythm and the prevention of recurrent episodes of AF/AFL in the canine sterile pericarditis model of postoperative AF/AFL. We also showed within our detailed cardiovascular safety assessment that GAP-134 neither inhibited the hERG potassium ion current nor produced any effects on AERP, heart rate, blood pressure, or the ECG. These findings suggest that GAP-134 may play a therapeutic role as a prophylactic treatment for postoperative AF/AFL in patients undergoing coronary artery bypass grafting and/or valve replacement surgeries. Furthermore, although our results are promising, future studies are needed to determine whether the ability of GAP-134 to maintain sinus rhythm can lead to a potential treatment for individuals who suffer from more chronic forms of AF, such as paroxysmal or persistent AF.

References


Address correspondence to: Dr. James K. Hennen, Cardiovascular and Metabolic Disease, Wyeth Research, Collegeville, PA 19426. E-mail: hennen@wyeth.com