Synergistic Electrophysiologic and Antiarrhythmic Effects of the Combination of Ranolazine and Chronic Amiodarone in Canine Atria

Serge Sicouri, MD; Alexander Burashnikov, PhD; Luiz Belardinelli, MD; Charles Antzelevitch, PhD, FAHA

Background—Amiodarone and ranolazine have been characterized as inactivated- and activated-state blockers of cardiac sodium channel current (INa), respectively, and shown to cause atrial-selective depression of INa-related parameters. This study tests the hypothesis that their combined actions synergistically depress INa-dependent parameters in atria but not ventricles.

Methods and Results—The effects of acute ranolazine (5 to 10 μmol/L) were studied in coronary-perfused right atrial and left ventricular wedge preparations and superfused left atrial pulmonary vein sleeves isolated from chronic amiodarone-treated (40 mg/kg daily for 6 weeks) and untreated dogs. Floating and standard microelectrode techniques were used to record transmembrane action potentials. When studied separately, acute ranolazine and chronic amiodarone caused atrial-predominant depression of INa-dependent parameters. Ranolazine produced a much greater reduction in Vmax and much greater increase in diastolic threshold of excitation and effective refractory period in atrial preparations isolated from amiodarone-treated versus untreated dogs, leading to a marked increase in postrepolarization refractoriness. The drug combination effectively suppressed triggered activity in pulmonary vein sleeves but produced relatively small changes in INa-dependent parameters in the ventricle. Acetylcholine (0.5 μmol/L) and burst pacing induced atrial fibrillation in 100% of control atria, 75% of ranolazine-treated (5 μmol/L) atria, 16% of atria from amiodarone-treated dogs, and in 0% of atria from amiodarone-treated dogs exposed to 5 μmol/L ranolazine.

Conclusions—The combination of chronic amiodarone and acute ranolazine produces a synergistic use-dependent depression of INa-dependent parameters in isolated canine atria, leading to a potent effect of the drug combination to prevent the induction of atrial fibrillation. (Circ Arrhythm Electrophysiol. 2010;3:88-95.)

Key Words: atrial fibrillation ■ antiarrhythmic drugs ■ sodium channel blocker ■ electrophysiology ■ pharmacology

Ranolazine is an antianginal agent recently shown to possess antiarrhythmic activity in ventricular and atrial myocytes, including pulmonary vein (PV) sleeve preparations.1-6 Chronic amiodarone is commonly used for the treatment of ventricular and supraventricular arrhythmias, including atrial fibrillation (AF).7,8

Clinical Perspective on p 95

Recent studies have demonstrated that amiodarone is an atrial-selective, inactivated-state blocker of cardiac sodium channel activity and that ranolazine is an atrial-selective, activated-state blocker of sodium channel activity.4,9 We hypothesized that the combination of ranolazine and chronic amiodarone would act synergistically to cause potent use-dependent depression of sodium channel current (INa)-dependent parameters in atrial but not ventricular tissues.

The present study was designed to determine the electrophysiological and antiarrhythmic effects of ranolazine in coronary-perfused right atrial and left ventricular preparations and superfused PV sleeves isolated from untreated and chronic amiodarone-treated dogs.

Methods

This investigation conforms to the Guide for Care and Use of Laboratory Animals published by the National Institutes of Health (National Institutes of Health publication No. 85-23, Revised 1996) and was approved by the Animal Care and Use Committee of the Masonic Medical Research Laboratory.

Adult mongrel dogs weighing 20 to 35 kg were anticoagulated with heparin (180 IU/kg) and anesthetized with sodium pentobarbital (35 mg/kg IV). The chest was opened via a left-thoracotomy and the heart excised and placed in a cold cardioplegic solution ([K+]o=12 mmol/L, 4°C).

Arterially Perfused Atrial and Ventricular Preparations

In vitro experiments were performed using isolated arterially perfused canine right atrial (RA) and left ventricular (LV) coronary-perfused wedge preparations (~3.0×1.2×1.2 cm). The methods used for isolation and perfusion of these preparations have been
described in previous publications. Briefly, the preparations were dissected from hearts removed from anesthetized (sodium pentobarbital) adult mongrel dogs (20 to 25 kg), untreated or treated with chronic amiodarone (40 mg/kg/d for 6 weeks). Unfolded RA with a rim of the right ventricle attached was perfused through the ostium of the right coronary artery and the LV wedge was perfused through a diagonal branch of the left anterior descending coronary artery. Unperfused tissue was removed with a razor blade or scissors. The cut ventricular and atrial coronary artery branches were ligated using silk thread. After these procedures (performed in cold cardioplegic solution, 4° to 8°C), the preparations were transferred to a temperature-controlled bath and arterially perfused with Tyrode solution by use of a roller pump at a rate of 8 to 10 mL/min. The composition of the Tyrode solution was (in mM): NaCl 129, KCl 4, NaH2PO4 0.9, NaHCO3 20, CaCl2 1.8, MgSO4 0.5, and D-glucose 5.5, buffered with 95% O2 and 5% CO2 (37° to 38°C, pH 7.35). Transmembrane action potential (AP) recordings were obtained using standard or floating glass microelectrodes. A pseudo-ECG was recorded using 2 electrodes consisting of Ag/AgCl half-cells placed in the Tyrode solution bathing the preparation, 1.0 to 1.2 cm from the 2 opposite sides of the atrial or ventricular coronary-perfused preparations. Diastolic threshold of excitation (DTE) was determined by increasing stimulus intensity in 0.01-mA steps. Effective refractory period (ERP) was measured by delivering premature stimuli at progressively shorter S1-S2 intervals after every 10th basic beat at a pacing cycle length (CL) of 500 ms (5-ms steps; 2 times the DTE). Postrepolarization refractoriness (PRR) was recognized when ERP exceeded action potential duration measured at 90% repolarization (APD90) in the ventricle and APD measured at 75% repolarization (APD75) in atria. Ventricular ERP was coincident with APD90, whereas atrial ERP was generally coincident with APD75.4 Stable action potential recordings could not be readily obtained in the vigorously contracting perfused preparations. Only action potentials having amplitudes of at least 100 mV were considered in the analysis. The largest recorded maximum rate of rise of the AP upstroke (Vmax) values per condition was taken for statistical analysis. The largest recorded maximum rate of rise of the AP upstroke (Vmax) could not be readily obtained in the preparations due to development of postrepolarization refractoriness. ERP increased to a greater degree than APD in both atria and ventricles, particularly in the atria, due to development of postrepolarization refractoriness. ERP were significantly longer in both atrial and ventricular preparations isolated from chronic amiodarone-treated dogs, compared with the respective tissues isolated from nontreated animals (Figure 1). Amiodarone-induced APD and ERP prolongation was greater in atria versus ventricles. ERP increased to a greater degree than APD in both atria and ventricles, particularly in the atria, due to development of postrepolarization refractoriness (PRR = ERP − APD).

Under control conditions, coronary-perfused atrial ERP was coincident with the APD value at 75% of repolarization (APD75), whereas ventricular ERP was coincident with APD75. APD and ERP were significantly longer in both atrial and ventricular preparations isolated from chronic amiodarone-treated dogs, compared with the respective tissues isolated from nontreated animals (Figure 1). Amiodarone-induced APD and ERP prolongation was greater in atria versus ventricles. ERP increased to a greater degree than APD in both atria and ventricles, particularly in the atria, due to development of postrepolarization refractoriness (PRR = ERP − APD).

Superfused PV Sleeve Preparation

PV sleeve preparations (approximately 2.0 × 1.5 cm) were isolated from canine left atria. The thickness of the preparation was approximately 2 mm. Left superior PVs were used in most experiments. The preparations were placed in a small tissue bath and superfused with Tyrode solution. PV preparations were stimulated at a basic cycle length (BCL) of 1000 ms during the equilibration period (1 hour) using electric stimuli of 1 to 3 ms duration and 2.5 times diastolic threshold intensity delivered through silver bipolar electrodes insulated except at the tips. Transmembrane potentials were recorded using glass microelectrodes filled with 2.7 mol/L KCl (10 to 20 mol/L Ω DC resistance) connected to a high input-impedance amplification system (World Precision Instruments, model KS-700, New Haven, Conn). Transmembrane action potentials were recorded at a sampling rate of 41 kHz.

Drugs

Amiodarone (Cordarone, 200 mg TAB) was obtained from Wyeth Pharmaceuticals, Vonore, Tenn, and was chronically administered orally at a dose of 40 mg/kg/d for a period of 6 weeks. Ranolazine (CV Therapeutics, now Gilead Sciences, Palo Alto, Calif) was used at concentrations of 5 and 10 μmol/L.

Statistics

Statistical analysis was performed using 1-way analysis of variance (ANOVA) for multiple groups or repeated-measures ANOVA followed by Bonferroni test as appropriate. The only exception was with comparison of changes in DTE because, in contrast to all the other data sets in which we report an n of 1 for each dog/preparation condition, DTE values in some cases had an n of 2 for each atria (ie, DTE was measured in both crista terminals and pectinate muscle regions of the right atrium). The following statistical approach was used for DTE comparison. To recognize that each preparation could have up to 4 measurements according to region and treatment with ranolazine, a mixed model including effects for region, chronic treatment with amiodarone (interparation comparison), before or after treatment with ranolazine (intrapreparation comparison), and interaction of treatment with amiodarone and ranolazine, and with repeated measurements for region and treatment with ranolazine was performed. We have used a completely general covariance matrix (unstructured for both region and treatment with ranolazine) and adopted the “Kenward Roger” option for degrees of freedom. The hypothesis of synergy corresponds to an interaction between the pretreatment of amiodarone versus placebo with before versus after treatment with ranolazine. The criterion for declaring statistically significant differences was P < 0.05, based on Bonferroni adjustment. All data are expressed as mean ± SD. The analysis of testing synergistic effect was conducted using software SAS 9.1.3 (SAS Institute Inc, Cary, NC), the Proc Mixed procedure.

Effects of Ranolazine on APD, ERP, and DTE

In atrial preparations (Figure 1A), addition of ranolazine (5 μmol/L) slightly prolonged APD(75) in either untreated controls (from 154±11 to 159±9 ms; P = 0.245) or chronic amiodarone atria (from 183±7 to 189±9 ms; P = 0.156) but significantly prolonged ERP in untreated (from 158±18 to 190±24 ms, P < 0.05) and chronic amiodarone (from 217±9 to 258±50 ms, P < 0.01). Ranolazine alone produced a 21-ms increase in PRR, whereas chronic amiodarone alone increased PRR by 39 ms. The combination of the two produced a synergistic effect, increasing PRR by 69 ms. In marked contrast, addition of ranolazine produced no significant change in either APD or ERP in ventricular wedge preparations isolated from untreated or chronic amiodarone-treated dogs (Figure 1B). Ventricular PRR did not increase at all with ranolazine alone and only modestly with the combination of ranolazine and chronic amiodarone treatment (28 ms). Both treatments alone or combined caused produced an atrial-preferential prolongation of ERP and PRR.

Ranolazine (5 μmol/L) reduced Vmax of atrial AP to a much greater extent in atrial preparations isolated from...
chronic amiodarone-treated dogs, compared with untreated animals (Figure 2). Ranolazine alone caused a small increase in DTE in endocardial pectinate muscle and crista terminals of the coronary-perfused atrial preparation (0.17±0.02 to 0.19±0.03 ms, Δ = 0.02 ms), chronic amiodarone increased DTE to 0.31±0.03 ms (Δ = 0.14 ms) and the 2 treatments combined produced a synergistic effect increasing DTE to 0.42±0.06 ms (Δ = 0.25 ms) (Figure 3) (P < 0.05, chronic amiodarone+ranolazine versus chronic amiodarone).

**Effects of Ranolazine on Activation Failure in Atrial Versus Ventricular Preparations**

In coronary-perfused atrial preparations, the shortest pacing CL permitting a 1:1 response was 129±8 ms in untreated controls, 221±39 ms with chronic amiodarone treatment, 234±29 ms after acute ranolazine treatment alone (5 μmol/L), and 325±34 ms after combined chronic amiodarone and ranolazine treatment (5 μmol/L) (P < 0.01 versus either treatment alone) (Figure 4 and the Table), reflecting reduced excitability and accentuated PRR. In the presence of acetylcholine (0.5 μmol/L), the shortest pacing CL permitting a 1:1 response was 71±12 ms in untreated controls, 136±22 ms with chronic amiodarone treatment, 94±31 ms with acute ranolazine treatment alone, and 205±34 ms with chronic amiodarone+ranolazine treatment.

In ACh-pretreated preparations, burst pacing induced AF in 100% of controls from untreated animals (10 of 10), in 16% (1 of 6) of atria isolated from chronic amiodarone-treated dogs, in 75% (3 of 4) of atria treated with ranolazine (5 μmol/L) only, and in 0% (0 of 4) in preparations with combined chronic amiodarone and ranolazine (5 μmol/L) treatments.

Similar depression of excitability was observed in PV sleeve preparations. Figure 5 shows use-dependent depression of Vmax and action potential in PV sleeves. The addition of 5 μmol/L ranolazine caused a marked reduction in Vmax and excitability resulting in 4:3 and 4:1 activation failure at CLs of 1000 and 300 ms. Addition of 10 μmol/L ranolazine produced complete activation failure in the PV sleeve preparation.

**Figure 1.** Effects of acute ranolazine, chronic amiodarone, and its combination on APD measured at 75% and 90% repolarization (APD75 and APD90) and ERP in coronary-perfused right atrial (A) and left ventricular wedge (B) preparations. Ranolazine significantly prolongs ERP but not APD in the atrium, causing significant PRR in atrial but not ventricular preparations isolated from chronic amiodarone-treated dogs. ERP−APD−PRR (n = 4 to 17), *P < 0.01 versus respective APD75 controls: Chronic amiodarone+ranolazine versus control, chronic amiodarone versus control. Atria: control, n = 17 dogs; ranolazine, n = 10 dogs; chronic amiodarone, n = 8 dogs; chronic amiodarone+ranolazine, n = 4 dogs. Ventricles: control, n = 5 dogs; ranolazine, n = 5 dogs; chronic amiodarone, n = 4 dogs; chronic amiodarone+ranolazine, n = 4 dogs.

**Figure 2.** Synergistic reduction of the maximum rate of rise of the action potential upstroke (Vmax) by combination of chronic amiodarone and acute ranolazine in canine coronary perfused RA preparations. Shown are Vmax values from individual experiments (n = 4 to 14). Control, n = 14 dogs; ranolazine, n = 10 dogs; chronic amiodarone, n = 14 dogs; chronic amiodarone+ranolazine, n = 4 dogs. Ran indicates ranolazine; Amio, chronic amiodarone. *P < 0.05 versus control and ranolazine 5 μmol/L.

**Figure 3.** Synergistic effects of acute ranolazine and chronic amiodarone to significantly increase DTE in coronary-perfused RA preparations (n = 6 to 12). Control, n = 9 recordings (8 PM and 1 CT) from 8 dogs; ranolazine, n = 9 recordings (8 PM and 1 CT) from 8 dogs; chronic amiodarone, n = 12 recordings (7 PM and 5 CT) from 8 dogs; chronic amiodarone+ranolazine, n = 6 recordings (4 PM and 2 CT) from 4 dogs. **P < 0.001 versus control; †P < 0.01 versus chronic amiodarone alone and versus ranolazine. PM indicates endocardial pectinate muscle, CT, crista terminalis.
Acceleration-induced activation failure was not observed in ventricular wedge preparations isolated from the LV wedge of chronic amiodarone-treated dogs either in the absence or presence of ranolazine (5 and 10 \(\mu\)mol/L; Figure 6).

The marked rate-dependent depression of INa in PV sleeve preparations, as reflected by the use-dependent decrease in \(V_{\text{max}}\), led to activation failure at slower rates in chronic amiodarone-treated preparations. This effect was much more accentuated in the presence of ranolazine; Figure 7 shows composite data of the effect of ranolazine on the BCL at which 1:1 activation failure first occurred in pulmonary vein sleeves isolated from untreated and chronic amiodarone-treated dogs. In untreated preparations, ranolazine (10 \(\mu\)mol/L) induced a significant increase in the BCL at which activation failure occurred in PV but not LV wedge preparations. Chronic amiodarone induced a significant increase in both PV sleeve and LV wedge preparations but a much greater increase in PV sleeves. The combination of chronic amiodarone and ranolazine produced a remarkable synergistic increase in the BCL, permitting 1:1 activation in PV sleeves, with little additional effect in the ventricular wedge preparations.

**Table.** Shortest \(S_1-S_1\) Permitting 1:1 Activation Under Control Conditions, After Chronic Amiodarone, Ranolazine, and the Combination of Chronic Amiodarone and Acute Ranolazine

<table>
<thead>
<tr>
<th>Condition</th>
<th>(S_1-S_1) (ms)</th>
<th>(S_1-S_1) + Acetylcholine (0.5 (\mu)mol/L)</th>
<th>(S_1-S_1) + Ranolazine (5 (\mu)mol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>129 ± 8</td>
<td>71 ± 12</td>
<td></td>
</tr>
<tr>
<td>Chronic amiodarone (n=8)</td>
<td>222 ± 39*</td>
<td>136 ± 22*</td>
<td></td>
</tr>
<tr>
<td>Ranolazine, 5 (\mu)mol/L (n=10)</td>
<td>227 ± 38*</td>
<td>94 ± 31*</td>
<td></td>
</tr>
<tr>
<td>Chronic amiodarone + ranolazine, 5 (\mu)mol/L (n=4)</td>
<td>325 ± 34†</td>
<td>205 ± 34†</td>
<td></td>
</tr>
</tbody>
</table>

*\(P<0.05\) versus respective controls: chronic amiodarone + ranolazine versus chronic amiodarone, ranolazine (5 \(\mu\)mol/L) versus control, and chronic amiodarone versus control.

†\(P<0.05\) versus ranolazine (5 \(\mu\)mol/L), chronic amiodarone, and control.

**Effects of Acute Ranolazine and Chronic Amiodarone on Triggered Activity Induced by Early Afterdepolarizations and Delayed Afterdepolarizations**

Previous studies have shown that ranolazine (10 \(\mu\)mol/L) alone suppresses late phase 3 early afterdepolarizations (EADs), delayed afterdepolarizations (DADs), and triggered activity elicited by exposure of the PV sleeves to ACh+isoproterenol, or high [Ca\(^{2+}\)]e+rapid pacing\(^6\) and that chronic amiodarone is capable of preventing the appearance of triggered activity (Figure 7A) and LV wedge (Figure 7B) preparations isolated from untreated and chronic amiodarone-treated dogs. In untreated preparations, ranolazine (10 \(\mu\)mol/L) induced a significant increase in the BCL at which activation failure occurred in PV but not LV wedge preparations. Chronic amiodarone induced a significant increase in both PV sleeve and LV wedge preparations but a much greater increase in PV sleeves. The combination of chronic amiodarone and ranolazine produced a remarkable synergistic increase in the BCL, permitting 1:1 activation in PV sleeves, with little additional effect in the ventricular wedge preparations.
of DADs, late phase 3 EADs, and triggered activity induced at fast rates in PV sleeves in 80% of PV sleeve preparations exposed to ACh, isoproterenol, high [Ca\textsuperscript{2+}]\textsubscript{o} or their combination.\textsuperscript{12} In the present study, neither DADs nor late phase 3 EADs were observed in PV sleeves in the presence of combined ranolazine and chronic amiodarone (n=6).

**Discussion**

The results of the present study indicate that the combination of chronic amiodarone and relatively low concentrations of acute ranolazine (therapeutic range, 2 to 8 μmol/L) produces a synergistic use-dependent depression of sodium channel-dependent parameters including V\textsubscript{max}, DTE, PRR, and 1:1 activation in isolated canine atria (coronary-perfused right atria and superfused left atrial PV sleeve preparations), leading to a much more potent effect of the drug combination to prevent the induction of AF. Importantly, LV preparations were much less affected by the drug combination. Altogether, the data show a marked atrial selectivity of combined acute ranolazine and chronic amiodarone to depress sodium channel-dependent parameters.

**Pharmacological and Electrophysiological Profile of Chronic Amiodarone and Ranolazine**

Amiodarone is widely used in the treatment of both supraventricular and ventricular arrhythmias. Its mechanism of action includes inhibition of a number of cardiac ionic currents (I\textsubscript{Kr}, I\textsubscript{Ks}, I\textsubscript{Na}, late I\textsubscript{Na}, I\textsubscript{to}, I\textsubscript{Ca-L}, I\textsubscript{Ca-T}, I\textsubscript{K1}, I\textsubscript{K(ACH)1}, I\textsubscript{K(ATP)}) as well as α- and β-adrenoceptor–blocking activity.\textsuperscript{8,13} Acute amiodarone inhibits I\textsubscript{Kr}, whereas chronic amiodarone also leads to a reduction of I\textsubscript{Ks}.\textsuperscript{14} Chronic amiodarone prolongs ventricular APD and QT interval.\textsuperscript{9,13,15} Both acute and chronic amiodarone have been shown to prolong the ERP more than APD, resulting in PRR.\textsuperscript{16,17} Whereas acute amiodarone reduces V\textsubscript{max} and blocks I\textsubscript{Na} in practically all studies (for review, see Reference 13),\textsuperscript{13} chronic amiodarone has been reported to either depress V\textsubscript{max} or to cause little to no change in V\textsubscript{max} in superfused ventricular muscle and Purkinje fibers.\textsuperscript{13,15,21} Ventricular conduction velocity is slowed and QRS duration is prolonged in both acute and chronic amiodarone-treated humans and animals in vivo.\textsuperscript{21–23}

Ranolazine has been shown to have a pharmacological profile similar to that of chronic amiodarone. Like amiodarone, ranolazine, although at different concentrations, causes inhibition of I\textsubscript{Na}, I\textsubscript{Kr}, and I\textsubscript{Ca}.\textsuperscript{12,24} Both agents have relatively rapid unbinding kinetics from the sodium channel (τ=1.56±0.56 and 0.3 to 1.6 seconds for ranolazine and chronic amiodarone, respectively).\textsuperscript{5,13} Like amiodarone, ranolazine produces an atrial-selective depression of I\textsubscript{Na}-dependent parameters. Unlike amiodarone, which is an inactivated-state blocker of cardiac sodium channels,\textsuperscript{13,25} ranolazine is an activated-state blocker.\textsuperscript{26} We hypothesized that an atrial-selective activated-state blocker such as ranolazine would potentiate the effects of atrial selective inactivated-state blocker such as amiodarone in producing use-dependent depression of I\textsubscript{Na}-mediated parameters and thus potentiate the effectiveness of amiodarone in the management of AF. Our results provide evidence in support of this hypothesis.

**Atrial-Selective Effects of Ranolazine and Chronic Amiodarone on Sodium Channel Activity**

The combination of rapid dissociation of drug from the sodium channel and an effect to preferentially prolong atrial APD, secondary to I\textsubscript{Kr} block, have been suggested to be key features for atrial-selective depression of sodium channel–dependent parameters and anti-AF efficacy.\textsuperscript{4,20}

Our data indicate that the combination of ranolazine and chronic amiodarone treatment produce an atrial-selective, use-dependent depression of V\textsubscript{max} and excitability that is much greater than either treatment alone and greater than the algebraic sum of the individual treatments, thus pointing to a synergism of the effects of the 2 therapies.\textsuperscript{4,6,9,20}

Under control conditions, take-off potential (TOP) of PV sleeves was −81±4, −80.6±4, −79.8±3, −77.6±3, and −75.6±3 mV at BCLs of 2000, 1000, 500, 300, and 200 ms, respectively. Thus, a change in BCL from 2000 to 200 ms results in a 5-mV depolarization of TOP. It is difficult to record a stable RMP from vigorously contracting coronary-
perfused LV wedge preparations. However, when stable recordings were obtained during a change in rate, no changes in RMP were observed between BCL 2000 and 20 ms. Thus, average RMP in LV is more hyperpolarized at 85.3 ± 2 mV and does not depolarize appreciably at rapid rates. The difference in behavior of TOP between atrial and ventricular tissues at fast rates is attributable to the much slower action potential phase 3 in atrial versus ventricular cells. A more positive TOP clearly contributes to the greater depression of atrial versus ventricular Vmax. We have previously shown that this contributes to the atrial selectivity of agents such as ranolazine and amiodarone.

The atrial-selective effects of both chronic amiodarone and ranolazine to depress peak INa and INa-dependent parameters are thought to result from the more negative steady-state inactivation relationship for INa as well as to the much slower action potential phase 3 of atrial versus ventricular cells. A more positive TOP clearly contributes to the greater depression of atrial versus ventricular Vmax. We have previously shown that this contributes to the atrial selectivity of agents such as ranolazine and amiodarone.

The slow phase 3 repolarization in atrial cells potentiates the effect of chronic amiodarone at rapid activation rates by abbreviating the diastolic interval and raising TOP of the next beat, thus reducing the availability of sodium channels and the number of channels in the rested state, from which the drug unbinds. Another factor that contributes to the reduced availability of peak INa in atria is the more depolarized RMP of atrial compared with ventricular cells. Intrinsic atrioventricular differences in RMP is due principally to a smaller IK1 in atrial versus ventricular cells.

Effects on EAD- and DAD-Induced Triggered Activity in PV Sleeves
Ectopic activity arising from the PV sleeves is thought to be an important source of triggers and in some cases substrate for the development of AF. Over the past decade, radiofrequency ablation has become the treatment of choice for drug-resistant AF. Segmental PV isolation and circumferential PV ablation are procedures now commonly used to suppress refractory atrial arrhythmias, including AF.

Our results suggest that combined ranolazine and chronic amiodarone prevent the appearance of DADs, late phase 3 EADs, and triggered activity induced in PV sleeves by exposure to ACh, isoproterenol, high [Ca2+]o, or their combination. Previous studies have shown that 10 μmol/L ranolazine can suppress EADs, DADs, or triggered activity in 100% (11 of 11) of PV sleeve preparations studied and that chronic amiodarone alone is capable of preventing the appearance of DADs, late phase 3 EADs, and triggered activity induced in PV sleeves in 80% of PV sleeve preparations. Additionally, chronic amiodarone was observed to prevent ACh-induced marked abbreviation of the action potential. The combination of ranolazine and chronic amiodarone was associated with a high degree of activation failure even at relatively slow rates. Neither EAD, DAD, or reentrant activity is likely to develop under these conditions.

Study Limitations
The dose of amiodarone used in the present study (40 mg/kg/d) is larger than that typically used in the clinic, where the loading dose of amiodarone ranges from 800 to 1600 mg/d (approximately 20 to 25 mg/kg/d). Similar dosage regimens have been used in previous studies, reflecting the relatively lower sensitivity of dogs to amiodarone. It should be noted, however, that loading doses are not given to patients for 6 weeks’ duration but typically for 1 to 2 weeks. Plasma or tissue concentrations of amiodarone were not measured in this study. However, tissue concentrations of amiodarone were measured in a previous study using similar doses of oral amiodarone in a dog experimental model. The tissue concentration of amiodarone in ventricular epicardium was 19.1 ± 8.1 ng/mL and remained largely unchanged over a 6-hour period. It is noteworthy that this is lower that the tissue concentration of amiodarone found in humans after chronic
amiodarone treatment (40 ng/mL) with a loading dose of 600 mg daily for 1 week, followed by 200 mg daily for a period of 7 weeks.\textsuperscript{33}

**Clinical Implications**

Most antiarrhythmic agents shown to be effective in terminating and/or preventing clinical AF or atrial flutter act primarily by reducing sodium current, $I_{Na}$ (eg, propafenone or flecaainide), or $I_{Kr}$ (eg, dofetilide) or by blocking multiple ion channels (amiodarone). Use of these agents is limited by their potential ventricular proarrhythmic actions and/or organ toxicity at therapeutically effective doses.\textsuperscript{2,34,35}

Acute and chronic amiodarone are widely used in the clinical management of atrial and ventricular arrhythmias.\textsuperscript{8,36,37} Chronic amiodarone is the most effective pharmacological agent available for the maintenance of sinus rhythm after termination of AF.\textsuperscript{8} The antiarrhythmic efficacy of chronic amiodarone has been attributed to the multiplicity of effects on ion channel activity (class I, II, III and IV actions), reduction of transmural dispersion of repolarization, induction of postrepolarization refractoriness, prolongation of the excitable gap, suppression of triggered activity, and inhibition of atrial remodeling.\textsuperscript{8,17,19,36,38,39}

Ranolazine has a pharmacological profile similar to that of chronic amiodarone.\textsuperscript{1,24} Although clinical trials of the actions of ranolazine to suppress AF are not available as yet, results of the MERLIN trial indicate that the drug shows both ventricular and supraventricular antiarrhythmic activity. Treatment with ranolazine resulted in significantly lower incidence of ventricular tachycardia lasting $\geq 8$ beats (5.3\% versus 8.3\%; $P=0.001$), supraventricular tachycardia (44.7\% versus 55.0\%; $P=0.001$), or new-onset AF (1.7\% versus 2.4\%; $P=0.08$).\textsuperscript{40}

Both amiodarone and ranolazine are atrial-selective in their actions. The synergism of their combined electrophysiological and anti-AF effects may be due to their interaction with different states of the cardiac sodium channel. The potentiation by ranolazine of the anti-AF effects amiodarone may permit the use of a lower dose of amiodarone to obtain a similar outcome, thus reducing its adverse effects that limit its clinical use.\textsuperscript{9}

Clinical and experimental studies have highlighted the role of PV in the triggering of atrial arrhythmias, AF in particular.\textsuperscript{41,42} In the present study, PV-selective depression of $I_{Na}$-related parameters was potentiated by addition of ranolazine to chronic amiodarone, leading to activation failure at relatively long CLs and complete suppression of triggered activity. The combined effect of chronic amiodarone and acute ranolazine suggest that ranolazine may help suppress AF in patients in whom amiodarone was not effective. The actions of chronic amiodarone plus ranolazine to produce potent block of the sodium channels in the atria are similar to that of class IC antiarrhythmic agents such as propafenone and flecaainide. However, unlike the IC agents, the electrophysiological effects of the drug combination is largely restricted to the atrial myocardium, rendering it atrial-selective.\textsuperscript{4,9} The drug combination is thus effective in terminating and preventing the reinduction of AF in experimental models of AF, without exerting an arrhythmogenic effect on ventricular myocardium, as is the case with putative sodium channel blockers such as propafenone.

We conclude that the combination of chronic amiodarone and relatively low concentrations of acute ranolazine produces a synergistic use-dependent depression of sodium channel–dependent parameters in isolated canine atria that lead to a potent effect of the drug combination to prevent the induction of AF.

**Acknowledgments**

We gratefully acknowledge the helpful advice of Jose M. Di Diego, MD, and the expert technical assistance of Judith Hefferon and Robert Goodrow, Jr. We are grateful to Whedy Wang, PhD, for statistical advice.

**Sources of Funding**

This study was supported by grant HL47678 from the National Heart, Lung, and Blood Institute and the New York State and Florida Grand Lodges of Free and Accepted Masons.

**Disclosures**

Dr Antzelevitch is a consultant to and received research support from CV Therapeutics (now Gilead Sciences, Inc). Dr Luiz Belardinelli is an employee of Gilead Sciences, Inc (Palo Alto, Calif).

**References**


CLINICAL PERSPECTIVE

Ranolazine is an antianginal agent recently shown to possess antiarrhythmic activity in ventricular and atrial tissues, including pulmonary vein sleeve preparations. Chronic amiodarone is commonly used for the treatment of ventricular and supraventricular arrhythmias, including atrial fibrillation. Ranolazine has been shown to have a pharmacological profile similar to that of chronic amiodarone. Like amiodarone, ranolazine produces inhibition of $I_{Na}$, $I_{Kr}$, and $I_{Ca}$. Both agents have relatively rapid unbinding kinetics from the sodium channel. Recent studies have demonstrated that amiodarone is an atrial-selective, inactivated-state blocker of cardiac sodium channel activity and that ranolazine is an atrial-selective, activated-state blocker of sodium channel activity. This study tests the hypothesis that the ranolazine and chronic amiodarone in combination synergistically depress sodium channel current in atrial but not ventricular tissues. The data presented demonstrate that the combination of chronic amiodarone and relatively low concentrations of acute ranolazine, within its therapeutic range, produces a synergistic atrial-selective use-dependent depression of sodium channel–dependent parameters that underlies a potent effect of the drug combination to prevent the induction of atrial fibrillation. These experimental data coupled with recent clinical observations suggest that additional studies specifically designed to evaluate the antiarrhythmic potential of combinations of atrial-selective sodium channel blockers may be warranted.
Synergistic Electrophysiologic and Antiarrhythmic Effects of the Combination of Ranolazine and Chronic Amiodarone in Canine Atria
Serge Sicouri, Alexander Burashnikov, Luiz Belardinelli and Charles Antzelevitch

_Circ Arrhythm Electrophysiol._ 2010;3:88-95; originally published online December 1, 2009; doi: 10.1161/CIRCEP.109.886275
_Circulation: Arrhythmia and Electrophysiology_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2009 American Heart Association, Inc. All rights reserved.
Print ISSN: 1941-3149. Online ISSN: 1941-3084

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circep.ahajournals.org/content/3/1/88

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation: Arrhythmia and Electrophysiology_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation: Arrhythmia and Electrophysiology_ is online at:
http://circep.ahajournals.org//subscriptions/