Electrophysiological Effects of Ranolazine, a Novel Antianginal Agent With Antiarrhythmic Properties

Charles Antzelevitch, PhD; Luiz Belardinelli, MD; Andrew C. Zygmunt, PhD; Alexander Burashnikov, PhD; José M. Di Diego, MD; Jeffrey M. Fish, DVM; Jonathan M. Cordeiro, PhD; George Thomas, PhD

Background—Ranolazine is a novel antianginal agent capable of producing antischismic effects at plasma concentrations of 2 to 6 μmol/L without reducing heart rate or blood pressure. The present study examines its electrophysiological effects in isolated canine ventricular myocytes, tissues, and arterially perfused left ventricular wedge preparations.

Methods and Results—Transmembrane action potentials (APs) from epicardial and midmyocardial (M) regions and a pseudo-ECG were recorded simultaneously from wedge preparations. APs were also recorded from epicardial and M tissues. Whole-cell currents were recorded from epicardial and M myocytes. Ranolazine inhibited \( I_{\text{Kr}} \) (IC\(_{50}\)=11.5 μmol/L), late \( I_{\text{Na}} \), late \( I_{\text{Ca,L}} \), peak \( I_{\text{Ca,L}} \), and \( I_{\text{Na,Ca}} \) (IC\(_{50}\)=5.9, 50, 296, and 91 μmol/L, respectively) and \( I_{\text{Ks}} \) (17% at 30 μmol/L), but caused little or no inhibition of \( I_{\text{Na,Ca}} \) or \( I_{\text{Ks}} \). In tissues and wedge preparations, ranolazine produced a concentration-dependent prolongation of AP duration of epicardial but abbreviation of that of M cells, leading to reduction or no change in transmural dispersion of repolarization (TDR). At [K\(^+\)]\(_{\text{e}}\)=4 mmol/L, 10 μmol/L ranolazine prolonged QT interval by 20 ms but did not increase TDR. Extrasystolic activity and spontaneous torsade de pointes (TdP) were never observed, and stimulation-induced TdP could not be induced at any concentration of ranolazine, either in normal or low [K\(^+\)]\(_{\text{e}}\). Ranolazine (5 to 20 μmol/L) suppressed early afterdepolarizations (EADs) and reduced the increase in TDR induced by the selective \( I_{\text{Ks}} \) blocker d-sotalol.

Conclusions—Ranolazine produces ion channel effects similar to those observed after chronic amiodarone (reduced \( I_{\text{Ks}} \), \( I_{\text{Kr}} \), late \( I_{\text{Na}} \), and \( I_{\text{Ca,L}} \)). The actions of ranolazine to suppress EADs and reduce TDR suggest that, in addition to its antianginal actions, the drug may possess antiarrhythmic activity. (Circulation. 2004;110:904-910.)

Key Words: ischemia ■ ion channels ■ intervals ■ depolarization

Results

Effect of Ranolazine on \( I_{\text{Kr}}, \ I_{\text{Ks}}, I_{\text{K1}}, I_{\text{to}} \), Late \( I_{\text{Na}}, \ I_{\text{Ca,L}}, \ I_{\text{Ca}}, \) and \( I_{\text{Na,Ca}} \)

Figures 1 to 3 show the effect of ranolazine to inhibit \( I_{\text{Ks}} \), late \( I_{\text{Na}} \), \( I_{\text{Ca,L}} \), late \( I_{\text{Ca}} \), and \( I_{\text{Na,Ca}} \) in canine left ventricular (LV) myocytes (midmyocardial and epicardial cells). Ranolazine inhibited both inward depolarizing and outward repolarizing currents. The potency (IC\(_{50}\)) of drug inhibition of late \( I_{\text{Na}} \) ranged between 5 and 21 μmol/L, with a greater potency at more positive voltage-clamp test potentials and higher frequency of depolarization. Although ranolazine inhibited late \( I_{\text{Ca,L}} \) with an IC\(_{50}\) of 50 μmol/L, significant inhibition (25% to 30%) occurred within the therapeutic range (2 to 6 μmol/L). The drug weakly inhibited \( I_{\text{Na,Ca}} \) (inward sodium-calcium exchange current), peak \( I_{\text{Ca,L}} \), and the slow component of the delayed rectifier potassium current (\( I_{\text{Ks}} \): 17% inhibition at 30 μmol/L and 20% at 900 μmol/L), but produced no effect on the outward rectifier potassium current (\( I_{\text{K}} \)) or the transverse outward potassium current (\( I_{\text{to}} \)).

Methods

See the online-only Data Supplement (listed with this article at http://www.circulationaha.org) for information about Methods.5,6

Received February 19, 2004; de novo received March 29, 2004; revision received May 12, 2004; accepted May 21, 2004.

From the Masonic Medical Research Laboratory, Utica, NY (C.A., A.C.Z., A.B., J.M.D., J.M.F., J.M.C., G.T.), and CV Therapeutics, Inc, Palo Alto, Calif (L.B.).

The online-only Data Supplement, which contains information about the Methods used in the study, can be found with this article at http://www.circulationaha.org.

Correspondence to Dr Charles Antzelevitch, Masonic Medical Research Laboratory, 2150 Bleecker St, Utica, NY 13501. E-mail ca@mmrl.edu

© 2004 American Heart Association, Inc.

Circulation is available at http://www.circulationaha.org

DOI: 10.1161/01.CIR.0000139333.83620.5D
Figure 3 shows the superimposed concentration-response curves for all of the currents for which such a relationship could be obtained. The plot highlights the overlap between the inhibition by ranolazine of $I_{Kr}$, late $I_{Na}$, and late $I_{Ca}$ at clinically relevant therapeutic concentrations (2 to 6 $\mu$mol/L), suggesting that the effect of the drug to block outward repolarizing current, which prolongs action potential duration (APD), is substantially counterbalanced by its effect to inhibit the inward current, which is expected to abbreviate the action potential.

**Effect of Ranolazine in Isolated Canine Ventricular Tissues**

Ranolazine caused no change in resting membrane potential, action potential amplitude, or overshoot in either midmyocardial (M) or epicardial cells, except at very high concen-
Figure 3. Summary of concentration-response relationships for effect of ranolazine to inhibit inward and outward ion channel currents in canine ventricular myocytes. Numbers inside parentheses are IC50 values for effect of ranolazine to inhibit rapidly activating delayed rectifier potassium current (I\textsubscript{Kr}), late sodium current (late I\textsubscript{Na}), peak calcium current (I\textsubscript{Ca}), late I\textsubscript{Ca}, and sodium-calcium exchange current (I\textsubscript{Na\textsubscript{Ca}}).

Table 1. Effects of Ranolazine on Phase 0 Amplitude, Resting Membrane Potential, and Overshoot of Action Potential in M Cell and Epicardial Preparations at a BCL of 500 ms

<table>
<thead>
<tr>
<th>Ranolazine Concentration, ( \mu \text{mol/L} )</th>
<th>Control</th>
<th>1</th>
<th>5</th>
<th>10</th>
<th>50</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>M cell</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amplitude</td>
<td>107±6</td>
<td>109±4</td>
<td>114±4</td>
<td>113±4</td>
<td>104±3</td>
<td>91±8*</td>
</tr>
<tr>
<td>RMP</td>
<td>-86±2</td>
<td>-86±1</td>
<td>-86±1</td>
<td>-88±1</td>
<td>-84±2</td>
<td>-82±3</td>
</tr>
<tr>
<td>Overshoot</td>
<td>21±6</td>
<td>23±5</td>
<td>27±3</td>
<td>25±3</td>
<td>19±1</td>
<td>9±6</td>
</tr>
<tr>
<td>Epicardial cell</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amplitude</td>
<td>95±2</td>
<td>93±2</td>
<td>101±1</td>
<td>94±2</td>
<td>86±6</td>
<td>93±2</td>
</tr>
<tr>
<td>RMP</td>
<td>-84±2</td>
<td>-84±2</td>
<td>-89±1</td>
<td>-88±1</td>
<td>-86±1</td>
<td>-85±2</td>
</tr>
<tr>
<td>Overshoot</td>
<td>11±1</td>
<td>10±2</td>
<td>12±1</td>
<td>8±2</td>
<td>0±6</td>
<td>8±4</td>
</tr>
</tbody>
</table>

RMP indicates resting membrane potential. All values are in mV, mean±SEM. n=5 for all. 
*P<0.05 vs control.
Effect of Ranolazine in Canine Left Ventricular Wedge Preparation

Table 2 shows the effect of ranolazine in isolated canine left ventricular wedge preparations. In the presence of 4 mmol/L [K\(^+\)]\(_o\) and at a BCL of 2000 ms, ranolazine caused a small concentration-dependent prolongation of epicardial APD and a small biphasic effect in the M region, resulting in a 30-ms QT prolongation at the highest concentration tested (100 μmol/L). It is noteworthy that much of the QT-interval prolongation observed at the higher concentrations of ranolazine is a result of a slowing of conduction secondary to sodium channel inhibition. Transmural dispersion of repolarization (TDR) showed a tendency to diminish, although the decrease was not statistically significant. Under hypokalemic conditions ([K\(^+\)]\(_o\)/3 mmol/L), ranolazine produced a much greater prolongation of the QT interval, but TDR showed a similar tendency to decrease.

Ranolazine did not induce either spontaneous or programmed electrical stimulation–mediated torsade de pointes (TdP) during endocardial or epicardial pacing of the canine left ventricular wedge preparation at any concentration up to 100 μmol/L. TdP did not develop, nor could it be induced, even in the presence of extremely low [K\(^+\)]\(_o\) (2 or 3 mmol/L) and high concentrations of ranolazine. In contrast, both spontaneous and stimulation-induced TdP have been shown to develop in the perfused wedge preparation in response to a wide variety of \(I_{Kr}\) blockers. 8

Neither early nor delayed afterdepolarizations were observed in either tissue or wedge preparations pretreated with any concentration of ranolazine. On the contrary, as illustrated in Figure 7, ranolazine proved to be effective in suppressing EADs in M cell and Purkinje fiber preparations pretreated with other \(I_{Kr}\) blockers, such as \(d\)-sotalol. \(d\)-Sotalol (100 μmol/L) produced a remarkable prolongation of repo-

![Figure 4](http://circ.ahajournals.org)  
**Figure 4.** Concentration-dependent effects of ranolazine on rate of rise of upstroke of a Purkinje fiber action potential (V\(_{max}\)). A, Superimposed action potentials and corresponding differentiated upstrokes (dV/dt) recorded in absence and presence of ranolazine (1 to 100 μmol/L) (BCL=500 ms). B, Concentration-response relationship of ranolazine’s effect to reduce V\(_{max}\). Data are presented as mean±SEM (n=5). C, Concentration-dependent effect of ranolazine on APD at 50% and 90% repolarization (APD\(_{50}\) and APD\(_{90}\), BCL=2000 ms. Values are mean±SEM. *P=0.05 vs control.

![Figure 5](http://circ.ahajournals.org)  
**Figure 5.** Left, 4 mmol/L [K\(^+\)]\(_o\). Effects of ranolazine on epicardial and M cell action potentials. A, Superimposed transmembrane action potentials recorded under control conditions and after addition of progressively higher concentrations of ranolazine (1 to 100 μmol/L). B, Concentration-response curves for effect of ranolazine on APD (APD\(_{50}\) and APD\(_{90}\)). Right, 2 mmol/L [K\(^+\)]\(_o\). Effects of ranolazine on epicardial and M cell action potentials recorded at a pacing cycle length of 2000 ms and [K\(^+\)]\(_o\) =2 mmol/L. C, Superimposed transmembrane action potentials recorded in absence and presence of ranolazine (1 to 100 μmol/L). D, Concentration-dependent effect of ranolazine on APD (APD\(_{50}\) and APD\(_{90}\), BCL=2000 ms. Data are presented as mean±SEM.
larization and induced EADs in both M cell and Purkinje fiber preparations. Ranolazine caused a concentration-dependent abbreviation of the action potential and abolished the EADs. A similar effect of ranolazine (5 to 20 μmol/L) to suppress EAD activity and abbreviate APD was observed in 4 of 4 M cell and 3 of 3 Purkinje fiber preparations. Moreover, ranolazine was found to be effective in reducing TD-APD, the interval between the peak and end of the T wave (Tpeak-Tend) and TDR under long-QT conditions (d-sotalol, moxifloxacin, and sea anemone toxin [ATX]-II) (data not shown).

Discussion
The effects of ranolazine on cardiac ion currents at concentrations within the therapeutic range (ie, 2 to 6 μmol/L) include inhibition of IKr, late INa, and late ICa,L. Inhibition of IKr by ranolazine prolongs APD, and its effect to inhibit late INa and late ICa,L abbreviates APD. The net effect and clinical consequence of inhibition of these ion channel currents is a modest increase in the mean QTc interval over the therapeutic range. The drug differs significantly from other agents that block IKr and induce TdP. Ranolazine-induced prolongation of the APD is rate independent (ie, does not display reverse

TABLE 2. Summary of the Effects of Ranolazine on the Action Potential Duration, QT Interval, and Transmural Dispersion of Repolarization in the Canine Arterially Perfused Left Ventricular Wedge Preparation

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Epicardium</th>
<th>M Region</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>APD50</td>
<td>APD90</td>
</tr>
<tr>
<td>4 mmol/L [K+]o (n=7 unless otherwise noted)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>218.0±7.5</td>
<td>270.3±9.6</td>
</tr>
<tr>
<td>1 μmol/L</td>
<td>228.6±12.9</td>
<td>285.9±14.3</td>
</tr>
<tr>
<td>5 μmol/L</td>
<td>228.6±12.9</td>
<td>285.9±14.3</td>
</tr>
<tr>
<td>10 μmol/L</td>
<td>250.6±10.8</td>
<td>298.4±13.7</td>
</tr>
<tr>
<td>100 μmol/L</td>
<td>250.6±10.8</td>
<td>298.4±13.7</td>
</tr>
</tbody>
</table>

| 3 mmol/L [K+]o (n=5 unless otherwise noted) | | | | | | |
| Control | 218.0±7.5 | 270.3±9.6 | 285.9±10.3* | 285.9±10.3 | 300.3±12.8* | 28.0±5.4 |
| 1 μmol/L | 228.6±12.9 | 285.9±14.3 | 285.9±14.3 | 285.9±14.3 | 293.0±12.6* | 29.5±7.7 |
| 5 μmol/L | 228.6±12.9 | 285.9±14.3 | 285.9±14.3 | 285.9±14.3 | 293.0±12.6* | 29.5±7.7 |
| 10 μmol/L | 250.6±10.8 | 298.4±13.7 | 298.4±13.7 | 298.4±13.7 | 314.4±11.5* | 26.0±5.4 |
| 100 μmol/L | 250.6±10.8 | 298.4±13.7 | 298.4±13.7 | 298.4±13.7 | 314.4±11.5* | 26.0±5.4 |

All values are in ms, mean±SEM. BCL=2000 ms.
*P<0.05 vs control.
†n=5.
‡n=3.
rate-dependent prolongation of APD) and is not associated with EADs, triggered activity, an increase in spatial dispersion of repolarization, or polymorphic ventricular tachycardia. Indeed, rather than displaying arrhythmogenic activity, ranolazine, via its actions to suppress EADs and reduce TDR, possesses significant antiarrhythmic activity, acting to suppress the arrhythmogenic effects induced by a variety of other QT-prolonging drugs.

Drugs with QT-prolonging properties have attracted considerable attention in recent years because of their proclivity to induce life-threatening cardiac arrhythmias, such as TdP.9–11 More than 50 commercially available or investigational noncardiovascular and 20 cardiovascular nonantiarrhythmic drugs have been shown or are suspected to have proarhythmic effects.

The mechanisms underlying TdP have long been a matter of debate. Recent studies have identified dispersion of repolarization secondary to accentuation of electrical heterogeneities intrinsic to ventricular myocardium as the substrate and EADs as the trigger for the development of TdP.8,10,12–15

Ventricular myocardium is composed of at least 3 electrophysiologically distinct cell types: epicardial, M, and endocardial. M cells are distinguished by having action potentials that prolong disproportionately relative to the action potentials of other ventricular myocardial cell types in response to a slowing of rate and/or in response to many QT-prolonging drugs.16–18 The ionic bases for these features include the presence of a smaller slowly activating delayed rectifier current (IKs),19 a larger late IKr20 and IKr,Ca21 IKr and IKs are similar in the 3 transmural cell types. M cells, like Purkinje fibers, develop EADs in response to agents and pathophysiological conditions that reduce the repolarization reserve of the ventricular myocardium. Epicardial and endocardial cells generally do not.

Most drugs that prolong the QT interval accentuate the normal transmural heterogeneity of final ventricular repolarization by causing a preferential prolongation of the action potential of M cells. IKr blockers, including d-sotalol, almokalant, E-4031, moxifloxacin, and erythromycin augment transmural dispersion of repolarization as a consequence. These agents cause relatively little prolongation of the APD of epicardial and endocardial cells, because these cell types possess a much more prominent IKs than the M cell. A similar preferential prolongation of the M cell APD is seen with agents that increase calcium current (IKc), such as Bay K 8644, as well as with agents that increase late IKr, such as ATX-II, anthopleurin-A, and DPI 201-106. An exception to this rule applies to agents that block IKs, which cause a similar percentage of APD prolongation in the 3 transmural cell types.

A more complex electrophysiological effect is observed with drugs affecting 2 or more ion channels, such as amiodarone, sodium pentobarbital, quinidine, cisapride, and azimilide. Amiodarone is a potent antiarrhythmic agent used in the management of both atrial and ventricular arrhythmias. In addition to its β-blocking properties, amiodarone is known to block late IKs,Ca, IKr, and IKs. The efficacy of the amiodarone and its low incidence of proarrhythmia relative to other agents with class III actions are attributable to this complex multichannel inhibition.22 When administered chronically, amiodarone increases QT without augmenting spatial dispersion of repolarization, unlike other IKs blockers.23–25 In some cases, transmural dispersion of repolarization is reduced.23 Chronic amiodarone therapy can also suppress the effect of other IKr blockers, like d-sotalol, to increase TDR or induce EADs.23 Thus, chronic amiodarone alters the cellular electrophysiology of ventricular myocardium so as to reduce TDR and suppress EADs, especially under conditions in which they are accentuated. The drug’s potent inhibition of late IKs is thought to play a key role.

The multichannel inhibition, particularly the ability to potently block late IKs, has been suggested to underlie the effect of IKr blockers to prolong QT without creating the substrate or trigger for the development of TdP. Indeed, this feature could contribute to the suppression of EADs and reduction of spatial dispersion of repolarization, the substrate and trigger for TdP. This is the case for amiodarone and sodium pentobarbital, as well as for high concentrations of quinidine and cisapride.24,26–28 Our data suggest that ranolazine fits this pharmacological profile as well. Like amiodarone and sodium pentobarbital, ranolazine produces a preferential prolongation of epicardial APDs, leading to a reduction in transmural dispersion of repolarization. The opposite effects of ranolazine on M cells and Purkinje fibers to that of epicardial APD is most likely because of the more prominent late IKs in the M cell and Purkinje fiber than in epicardial cells.20 Ranolazine is among the most potent late
block late $I_{Na}$ and Purkinje fiber but a decrease in net outward current in epicardium. The effect of ranolazine to block late $I_{Na}$, most likely underlies its effect to suppress EAD activity. Thus, unlike other $I_{Na}$ blockers, ranolazine does not lead to the development of TdP, either spontaneous or stimulation induced. Of note, ranolazine has recently been evaluated in an anesthetized dog model with acute complete atioventricular block, a model susceptible to drug-induced polymorphic ventricular tachycardia. At doses that prolonged the QT interval by approximately 5% to 11% above control, ranolazine did not cause spontaneous TdP or TdP facilitated by an intravenous bolus of phenylephrine (which increases susceptibility to TdP) in 5 dogs, whereas sotalol induced TdP in all 5 dogs under these conditions.29 Recent studies involving isolated guinea pig and rabbit hearts have also reported failure of ranolazine to induce TdP but its effectiveness to suppress TdP induced by selective $I_{Na}$ blockers (E-4031) and agents that augment late $I_{Na}$ (ATX-II).29 In summary, the available data suggest that ranolazine, in addition to its antiarrhythmic actions, may possess important antiarrhythmic activity.

Acknowledgments

This study was supported by grant HL-47678 from the National Heart, Lung, and Blood Institute (Dr Antzelevitch) and grants from the American Heart Association (Drs Burashnikov, Fish, and Antzelevitch), CV Therapeutics (Dr Antzelevitch), and the NY State and Florida Grand Lodges of the Free and Accepted Masons.

References

Electrophysiological Effects of Ranolazine, a Novel Antianginal Agent With Antiarrhythmic Properties
Charles Antzelevitch, Luiz Belardinelli, Andrew C. Zygmunt, Alexander Burashnikov, José M. Di Diego, Jeffrey M. Fish, Jonathan M. Cordeiro and George Thomas

_Circulation_. 2004;110:904-910; originally published online August 9, 2004;
doi: 10.1161/01.CIR.0000139333.83620.5D
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/110/8/904

Data Supplement (unedited) at:
http://circ.ahajournals.org/content/suppl/2004/08/23/01.CIR.0000139333.83620.5D.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ 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_ is online at:
http://circ.ahajournals.org/subscriptions/