Antitorsadogenic Effects of (±)-N-(2,6-Dimethyl-phenyl)-(4[2-hydroxy-3-(2-methoxyphenoxy)propyl]-1-piperazine (Ranolazine) in Anesthetized Rabbits

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ABSTRACT

Ranolazine [Ranexa; (±)-N-(2,6-dimethyl-phenyl)-(4[2-hydroxy-3-(2-methoxyphenoxy)propyl]-1-piperazine] is novel anti-ischemic agent that has been shown to inhibit late INa and IKr and to have antiarrhythmic effects in various preclinical in vitro models. This study was undertaken to investigate the effects of ranolazine on drug-induced Torsade de Pointes (TdP) in vivo. TdP was induced by an INa blocker, clofilium, in anesthetized, α1-agonist-sensitized rabbits. Clofilium prolonged QT interval corrected for heart rate (QTc) (52 ± 9%) and monophasic action potential duration (MAPD)90 (56 ± 9%) and caused TdP in eight of eight rabbits. Pretreatment with ranolazine (480 μg/kg/min) or lidocaine (200 μg/kg/min) reduced the clofilium-induced prolongation of QTc (15 ± 3 and 19 ± 3%, respectively, p < 0.001 versus vehicle) and MAPD90 (21 ± 4 and 20 ± 2%, respectively, p < 0.001 versus vehicle) and prevented the occurrence of TdP (zero of eight and zero of eight, respectively). Administration of ranolazine after the first episode of TdP terminated TdP and prevented its recurrence (zero of four versus vehicle, four of four). To rule out an α1-adrenoceptor antagonistic activity of ranolazine, we compared the effects of ranolazine on blood pressure with those of the α1-agonist, prazosin. Although prazosin (10 μg/kg/min) markedly shifted the phenylephrine (α1-agonist) dose-response curve to the right, it did not have any effect on clofilium-induced prolongation of QTc and MAPD90 (43 ± 7 and 53 ± 9%, respectively) or the occurrence of TdP (seven of eight). In contrast, ranolazine completely suppressed TdP but did not cause any shift in the phenylephrine dose-response curve at the highest dose tested (480 μg/kg/min). We conclude that ranolazine antagonizes the ventricular repolarization changes caused by clofilium and suppresses clofilium-induced TdP in rabbits.

Ranolazine (Ranexa) is a novel anti-ischemic agent that does not cause clinically significant hemodynamic effects (i.e., hypotension) and bradycardia (Chaitman et al., 2004). Results of nonclinical electrophysiological studies revealed that ranolazine affects various ion currents in cardiomyocytes. Within the therapeutic plasma concentration range (2–8 μM), the electrophysiological effects of ranolazine are probably due to the inhibition of late INa and IKr, with potencies (IC50 values) of 6 and 12 μM, respectively. At a higher concentration (IC50 = 50 μM), ranolazine also reduces late INa,1.5 (Antzelevitch et al., 2004b). In atrial myocytes, inhibition of peak INa probably contributes to the electrophysiological effect of ranolazine (Burashnikov et al., 2007). In ventricular myocytes from an LQT-3 mouse with mutant (ΔKPQ) Nav1.5 channels, ranolazine was found to be approximately 9 times more potent at blocking late Na+ than peak Na+ current (Fredj et al., 2006), whereas in canine ventricular myocytes from failing hearts, the late versus peak INa selectivity was 38-fold (Undrovinas et al., 2006).

Inhibition of pharmacologically or pathologically enhanced late INa by ranolazine has been proposed to explain its reported antiarrhythmic effects in various cardiac preparations (Antzelevitch and Belardinelli, 2006; Makielski and Valdivia, 2006). Inhibition of late INa by ranolazine shortens the ventricular action potential duration (APD) and thus offsets the potential proarrhythmic effect of APD prolongation caused by inhibition of INa. Another potential benefit of inhibiting late INa is the reduction of intracellular Na+ concentration and [Na+]i-dependent Ca2+ overload, an effect that should contribute to an improved intracellular Na+ and Ca2+ homeostasis and electrical stability.

ABBREVIATIONS: ranolazine, (±)-N-(2,6-dimethyl-phenyl)-(4[2-hydroxy-3-(2-methoxyphenoxy)propyl]-1-piperazine; APD, action potential duration; TdP, Torsade de Pointes; BP, blood pressure; MAP, monophasic action potential; ECG, electrocardiogram; HR, heart rate; ANOVA, analysis of variance; MAPD, monophasic action potential duration; EAD, early afterdepolarization; E-4031, [phenyl-14C]4-[1-[2-(6-methyl-2-pyridyl)ethyl]-4-piperidyl]carbonyl]-methanesulfanamide; PR, the ECG interval from the beginning of the P-wave to the peak of the R-wave; QRS, the ECG interval from the beginning of the Q-wave to the end of the S-wave; QT, the ECG QT interval corrected for heart rate; RR, the ECG interval between two consecutive R-waves.
Materials and Methods

Animal Surgical Preparation. Female New Zealand rabbits (3.0–4.0 kg) were purchased from Western Oregon Rabbit Company (Philomath, OR). Animals were housed on a 12-h light/dark cycle and received standard laboratory chow and water ad libitum. All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by National Research Council and with the experimental procedure approved by the Institutional Animal Care Committee of CV Therapeutics, Inc.

Rabbits were anesthetized with i.m. ketamine (35 mg/kg) and xylazine (5 mg/kg), with additional anesthetic given if necessary. A tracheotomy was performed, and the trachea was intubated with an endotracheal tube. The animal was artificially ventilated with room air supplemented with oxygen using a pressure control animal ventilator (Kent Scientific Corp., Torrington, CT) at a respiratory rate of 40 strokes/min and peak inspiration pressure of 10 mmH2O, which was adjusted to keep blood gases and pH within the physiological range (iSTAT clinic analyzer; Heska Corp., Waukesha, WI). External jugular and femoral veins were cannulated for drug administration. The femoral artery was cannulated for the measurement of blood pressure (BP). An electrode catheter (Irvine Biomedical Inc., Irvine, CA) was placed in the left ventricle via the right carotid artery and was gently pushed against the free wall of the ventricle to obtain monophasic action potential (MAP). Needle electrodes were inserted s.c. into the limbs for recording of the surface electrocardiogram (ECG). The BP, ECG, and MAP were monitored and recorded continuously with a computer data acquisition system (PowerLab; AD Instruments, Mountain View, CA). The body temperature of the animal was monitored via a rectal thermometer and maintained at 37 to 39°C by adjusting the surface temperature of the surgical table.

Induction of Torsade de Pointes. Animals were randomly assigned to six groups (n = 8, each group): vehicle (0.5% ascorbic acid in saline), lidocaine (200 μg/kg/min), prazosin (10 μg/kg/min), and three dose groups of ranolazine (120, 240, and 480 μg/kg/min). The dose regimens for ranolazine were targeted to achieve plasma concentrations between 5 and 24 μM. This concentration range covers the clinically therapeutic concentrations (mid to high end) as well as the concentrations that could be proarrhythmic (equal to or higher than IC50 of 12 μM for IKr blockade). The plasma concentrations of ranolazine were determined by using a high-performance liquid chromatography-tandem mass spectrometric assay. The plasma concentrations were determined to be 7–14, and 28 μM at 10 min after infusions of ranolazine at 120, 240, and 480 μg/kg/min, respectively (n = 6). Baseline measurements of BP, heart rate (HR), ECG, and MAP were obtained over a period of 1 to 2 min after 10 min of postsurgical stabilization. TdP was induced as previously described by Carlsson et al. (1990). In brief, methoxamine (15 μg/kg/min) was infused i.v. for 10 min followed by clofilium (100 nmol/kg/min). Both drugs were then continuously infused for the duration of the experiment (1 h after initiation of the clofilium infusion) or until the occurrence of an irreversible fatal arrhythmia (TdP degenerated to fatal ventricular fibrillation). In a randomized and blinded manner, ranolazine, lidocaine, prazosin, or vehicle was infused i.v. 10 min before clofilium and throughout the duration of the experiment.

In another set of experiments (acute intervention), animals were randomly divided into two groups: vehicle and ranolazine (n = 4, each group). To rule out the possibility of methoxamine-specific effects, another α1-agonist, phenylephrine (10 μg/kg/min), was used to facilitate induction of TdP by clofilium. Immediately after the appearance of the first episode of TdP, ranolazine was injected i.v. bolus (6 mg/kg) followed by i.v. infusion (480 μg/kg/min; plasma concentration, –28 μM) for 30 min. For vehicle control animals, 0.5% ascorbic acid was administered at the same rate. At that time, the infusion of clofilium and phenylephrine were stopped. The recurrence of TdP was examined during the 30-min infusion of ranolazine or vehicle. α1-Antagonistic Effect. Because an α1-adrenoceptor agonist (methoxamine or phenylephrine) is necessary for the induction of TdP in Carlson’s model (Carlsson et al., 1990), and ranolazine has been reported to have α1-adrenoceptor antagonistic activity, the potential α1-adrenoceptor antagonistic activity of ranolazine was investigated over a wide concentration range, from therapeutic concentration for angina (2–8 μM) to almost 90% inhibition of IC50 (30 μM), and compared with that of prazosin. Animals were randomly assigned to five groups (n = 5, each group): vehicle (0.5% ascorbic acid in saline), two dose groups of prazosin (0.5 and 5 μg/kg/min), and two dose groups of ranolazine (60 and 480 μg/kg/min). In the two dose groups of ranolazine (60 and 480 μg/kg/min), plasma concentration at 10 min after initiation of infusion were –3.5 and 28 μM, respectively. A short-acting α1-adrenoceptor agonist phenylephrine was used in this experiment. Phenylephrine dose-response curves for the diastolic BP, which reflects the α1-adrenoceptor-mediated vasconstriction, in the absence and presence of ranolazine or prazosin were constructed. Ranolazine, prazosin, or vehicle was infused i.v. after recording baseline BP and ECG. Ten minutes later, the animal was challenged with increasing doses of phenylephrine (0.3–300 μg/kg i.v.). The dose increment for phenylephrine was 3 times the previous dose, and the dosing interval was 3 to 5 min or longer at high doses, allowing BP to return to almost baseline level.

Chemicals and Reagents. Ranolazine was synthesized by CV Therapeutics, Inc. and was dissolved in 0.5% ascorbic acid in saline. Clofilium was purchased from Alexis Corporation (Lausen, Switzerland). Methoxamine, phenylephrine, and prazosin were purchased from Sigma-Aldrich (St. Louis, MO). All drugs were dissolved in saline, except prazosin, which was dissolved in distilled water.

Data Analysis. The QT interval was corrected for HR by using Carlson’s formula developed for rabbits: QTc = QT − 0.175 × (RR−300). Mean BP was calculated as 1/3 systolic + 2/3 diastolic blood pressure. All data are expressed as mean ± S.E.M. for group size “n.” Two-way ANOVA was performed followed by post hoc comparison of Bonferroni’s test unless indicated. Fisher’s exact test was applied for proportion data (incidence). The criterion for statistical significance was chosen as p < 0.05.

Results

Effect of Ranolazine on Hemodynamics and ECG Intervals. Table 1 summarizes the effect of ranolazine or prazosin alone on hemodynamics and ECG intervals. Ranolazine had no significant effect on HR or PR or QRS intervals, but at the highest dose (480 μg/kg/min), it caused a significant decrease in mean BP (21 ± 3%) and increase in QT (9 ± 2%) and QTc (12 ± 1%) intervals (Table 1).

Prevention of TdP by Ranolazine, Lidocaine, and Prazosin. The baseline values for mean BP, HR, and ECG intervals and MAPDmin were not different among vehicle, prazosin, lidocaine, and the three doses of ranolazine (Table 2). In the
and ECG R-on-T phenomena occurred in all eight animals, i.v. infusion of clofilium. Early afterdepolarizations (EADs) and TdP occurred in all eight rabbits at mean onset times of mature ventricular contractions, ventricular tachycardia, and other ECG intervals (Figs. 1 and 2, vehicle group). Pre- cinctly prolonged QT, QTc, and MAPD90 (48 sin, lidocaine, or ranolazine. Subsequently, clofilium signifi- 

**TABLE 1**

Effect of ranolazine and prazosin on hemodynamics and ECG intervals in rabbits

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (µg/kg/min)</th>
<th>n</th>
<th>Mean BP (mm Hg)</th>
<th>HR (beats/min)</th>
<th>PR (ms)</th>
<th>QRS (ms)</th>
<th>QT (ms)</th>
<th>QTc (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>0</td>
<td>5</td>
<td>69 ± 2</td>
<td>197 ± 14</td>
<td>78 ± 4</td>
<td>32 ± 2</td>
<td>146 ± 6</td>
<td>146 ± 4</td>
</tr>
<tr>
<td>Prazosin</td>
<td>0.5</td>
<td>5</td>
<td>71 ± 1*</td>
<td>200 ± 17</td>
<td>75 ± 3</td>
<td>33 ± 2</td>
<td>152 ± 6</td>
<td>149 ± 3</td>
</tr>
<tr>
<td>Ranolazine</td>
<td>480</td>
<td>5</td>
<td>Pre-</td>
<td>72 ± 2</td>
<td>82 ± 3</td>
<td>34 ± 2</td>
<td>155 ± 5</td>
<td>147 ± 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Post-</td>
<td>68 ± 2</td>
<td>80 ± 3</td>
<td>33 ± 2</td>
<td>154 ± 4</td>
<td>145 ± 3</td>
</tr>
</tbody>
</table>

* p < 0.05 compared with predrug (two-way repeated ANOVA followed by Bonferroni’s test).

**TABLE 2**

Baseline hemodynamic and electrophysiological parameters in various groups

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Dose (µg/kg/min)</th>
<th>n</th>
<th>Mean BP (mm Hg)</th>
<th>HR (beats/min)</th>
<th>PR (ms)</th>
<th>QRS (ms)</th>
<th>QT (ms)</th>
<th>QTc (ms)</th>
<th>MAPD90 (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>10</td>
<td>8</td>
<td>65 ± 2</td>
<td>185 ± 10</td>
<td>80 ± 2</td>
<td>32 ± 2</td>
<td>157 ± 7</td>
<td>152 ± 5</td>
<td>129 ± 4</td>
</tr>
<tr>
<td>Prazosin</td>
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<td>8</td>
<td>68 ± 3</td>
<td>174 ± 12</td>
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<td>35 ± 6</td>
<td>163 ± 4</td>
<td>153 ± 2</td>
<td>134 ± 5</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>5</td>
<td>8</td>
<td>66 ± 1</td>
<td>180 ± 5</td>
<td>79 ± 2</td>
<td>39 ± 1</td>
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<td>157 ± 3</td>
<td>132 ± 3</td>
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<td>Ranolazine</td>
<td>120</td>
<td>8</td>
<td>Pre-</td>
<td>67 ± 2</td>
<td>77 ± 2</td>
<td>32 ± 1</td>
<td>159 ± 5</td>
<td>152 ± 4</td>
<td>128 ± 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Post-</td>
<td>69 ± 1</td>
<td>77 ± 3</td>
<td>34 ± 1</td>
<td>161 ± 3</td>
<td>154 ± 3</td>
<td>130 ± 3</td>
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<td>Pre-</td>
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<td>79 ± 3</td>
<td>36 ± 2</td>
<td>155 ± 7</td>
<td>150 ± 4</td>
<td>130 ± 5</td>
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</tbody>
</table>

vehicle group, methoxamine infusion markedly increased mean BP (30 ± 4%) and correspondingly decreased HR (30 ± 5%) (Fig. 1). The QT interval and MAPD90 were slightly prolonged by methoxamine (7 ± 2 and 11 ± 4%, respectively) but did not reach statistical significance (Fig. 2). Correcting the QT interval with HR (RR interval) diminished the apparent prolongation of QT interval (QTc change, −10 ± 3%), indicating that the prolongation was in part due to the slowing of HR caused by methoxamine (Fig. 2). PR and QRS intervals were not affected by methoxamine (2 ± 2 and 3 ± 2%, respectively).

Treatment with prazosin and ranolazine reduced methox- amine-induced mean BP elevation and HR reduction (Fig. 1). Prazosin, but not ranolazine, tended to inhibit methoxamine- induced prolongations in QT and MAPD90 (0.3 ± 0.7 and −2 ± 1%, respectively), but these effects were not statisti- cally significant and might be attributed to the changes in HR (QTc, −4 ± 1% versus vehicle control, −10 ± 3%). The HR-corrected QT interval (QTc) was prolonged by ranolazine at 480 µg/kg/min (QTc, 8 ± 1%). Prazosin had no statistically significant effects on methoxamine-induced changes in mean BP, HR, and ECG intervals and MAPD90 (Fig. 2).

In the presence of methoxamine alone, no arrhythmias were observed during the 10-min infusion of vehicle, prazo- sin, lidocaine, or ranolazine. Subsequently, clofilium signifi- cantly prolonged QT, QTc, and MAPD90 (48 ± 7, 52 ± 9, and 56 ± 9%, respectively) without a significant effect on HR, BP, and other ECG intervals (Figs. 1 and 2, vehicle group). Pre- mature ventricular contractions, ventricular tachycardia, and TdP occurred in all eight rabbits at mean onset times of 6 ± 1, 6 ± 1, and 7 ± 2 min, respectively, after the start of the i.v. infusion of clofilium. Early afterdepolarizations (EADs) and ECG R-on-T phenomena occurred in all eight animals, and short-long-short R-R sequences were seen in five of eight animals.

As illustrated in the representative ECG and MAP trac- ings of Fig. 3, ranolazine and lidocaine, but not prazosin, reduced clofilium-induced QTc and MAPD90 prolongation. The occurrence of TdP was prevented by ranolazine in a dose-dependent manner with an ED90 value of 188 µg/kg/ min. Ranolazine at 480 µg/kg/min and lidocaine at 200 µg/ kg/min completely prevented the induction of TdP by clofi- lium (zero of eight, p < 0.001 versus vehicle control, eight of eight) (Fig. 4). Ranolazine at 120 µg/kg/min and prazosin at 10 µg/kg/min did not reduce the incidence of TdP (six of eight and seven of eight, respectively, versus vehicle control, eight of eight). However, the onset of TdP was delayed by treat- ment with prazosin (mean onset time, 23 ± 4 min versus vehicle control, 7 ± 2 min, p < 0.05 via one-way ANOVA, nonparametric analysis). There was a trend to delay the onset of TdP by ranolazine at 120 µg/kg/min, but the effect was not statistically significant (19 ± 8 min versus vehicle control, 7 ± 2 min).

**Termination of TdP by Ranolazine.** In another series of experiments (acute intervention), phenylephrine was used to facilitate the induction of TdP by clofilium, and the results were similar to those seen with methoxamine. There was an increase in mean BP and a decrease in HR (68 ± 9 and −40 ± 3%, respectively) by phenylephrine and prolongation in QT, QTc intervals, and MAPD90 (24 ± 5, 36 ± 6, and 23 ± 4%, respectively) upon addition of clofilium. TdP was induced in all eight rabbits. Recurrent episodes (16 ± 5 in 30 min) of TdP were seen in four of four rabbits in the vehicle group. However, in four rabbits treated with ranolazine (6 mg/kg i.v. bolus followed by a maintenance dose of 480 µg/kg/min), TdP
was immediately terminated, and recurrence of TdP was prevented (zero of four versus vehicle control, four of four, \( p < 0.05 \)).

\( \alpha_1 \)-Adrenoceptor Antagonistic Effect of Ranolazine and Prazosin. Phenylephrine dose-dependently increased the diastolic BP. As shown in Fig. 5, prazosin at 5 \( \mu \)g/kg/min, which is half of the dose given to prevent TdP, caused a 6-fold rightward parallel shift of the phenylephrine dose-response curve (ED\(_{50} \), 310 \( \pm \) 1 \( \mu \)g/kg versus vehicle control, 50 \( \pm \) 1 \( \mu \)g/kg). In contrast, ranolazine did not cause a significant shift of the phenylephrine dose response, even at the highest dose tested (480 \( \mu \)g/kg/min), a dose at which ranolazine completely suppressed TdP (ED\(_{50} \) 56 \( \pm \) 1 \( \mu \)g/kg versus vehicle control 50 \( \pm \) 1 \( \mu \)g/kg) (Fig. 5).

Discussion

The present study was designed to investigate the anti-arrhythmic effect of ranolazine in vivo using a drug-induced TdP model in rabbits. The data show that ranolazine treatment prevents, as well as terminates, clofilium-induced TdP, demonstrating that ranolazine has antiarrhythmic activity. In addition, the data show that ranolazine is not proarrhythmic, even though it prolongs MAPD and QTc intervals at the highest concentration tested. These results are in agreement with those of previous in vitro studies, which showed that ranolazine suppresses EADs and ventricular tachyarrhythmias induced by \( I_{Ks} \) blockers (E4031, \( \Delta \)-sotalol, and cisapride) in rabbit isolated hearts (Antzelevitch and Belardinelli, 2006).
The anti-TdP effect of ranolazine and lidocaine is associated with attenuation of the clofilium-induced prolongation in ventricular repolarization (manifested as MAPD and QTc in this study) (Fig. 2). Ranolazine and lidocaine both interact with Na\(^+\) channels at the same site on domain IV S6 (Fredj et al., 2006) and preferentially block the late Na current (Wasserstrom and Salata, 1988; An et al., 1996; Dumaine and Kirsch, 1998; Antzelevitch et al., 2004b). Because late INa plays a prominent role in determining APD (Gintant et al., 1984; Carmeliet, 1987; Kiyosue and Arita, 1989; Maltsev et al., 1998; Sakmann et al., 2000) and a facilitating role in EAD formation, particularly under conditions in which IKr and IKs are reduced (Clancy and Rudy, 1999; Antzelevitch, 2000; Song et al., 2004; Fedida et al., 2006; Orth et al., 2006), it is reasonable to attribute the anti-TdP effect of ranolazine to its late INa blockade. By this hypothesis, blocking late INa with ranolazine and lidocaine offsets the prolongation of ventricular repolarization by the IKr blocker clofilium and thereby inhibits clofilium-induced TdP. In addition, during prolonged repolarization with an IKr blocker, the intracellular Ca\(^{2+}\) rises, which may elicit EAD and TdP (Choi et al., 2002; Clusin, 2003). The blockade of late INa decreases intracellular Na\(^+\), thereby decreasing intracellular Ca\(^{2+}\) concentration via the Na\(^+\)/Ca\(^{2+}\) exchanger. In other words, the late INa-blocking effect of ranolazine is expected to improve intracellular Ca\(^{2+}\) homeostasis and promote electrical stability (Belardinelli et al., 2006; Noble and Noble, 2006). Based on the above consideration, we propose that the late INa-blocking effect is a main mechanism underlying antiarrhythmic effect of ranolazine in this model.

Ranolazine has been shown to bind to \(\alpha_1\)-adrenoceptors in various rat tissues and also attenuates phenylephrine evoked responses in the rat (Allely et al., 1993; W. Q. Wang, A. K. Dhall, and L. Belardinelli, unpublished data on file at CVT). Because an \(\alpha_1\)-adrenoreceptor agonist is necessary for the in-
duction of TdP in this model (Carlsson et al., 1990), to rule out a potential role for an α₁-adrenoceptor antagonistic effect of ranolazine in preventing TdP, we compared the antiarrhythmic versus α₁-antagonistic effect of ranolazine with that of the prototypical α₁-adrenoceptor antagonist prazosin. Ranolazine completely prevented the occurrence of TdP (Fig. 4) at a dose (480 μg/kg/min; plasma concentration, ~28 μM) that did not exhibit a significant α₁-antagonistic effect on peripheral arterioles (vasoconstriction) (Fig. 5). In contrast, prazosin did not produce marked anti-TdP effects (Fig. 4) at a dose (10 μg/kg/min) twice as high as the dose (5 μg/kg/min) that caused a significant shift of the phenylephrine dose-response curve (Fig. 5). Therefore, we conclude that an α₁-adrenoceptor antagonistic effect is not the major mechanism underlying the anti-TdP action of ranolazine.

Although Carlsson et al. (1990) have reported that prazosin had anti-TdP effects in this rabbit model, the dose of prazosin in that study was 50 times higher than that used in the present study. The observation time was also shorter (30 min) compared with the present study (60 min), which may have underestimated the occurrence of TdP because we noted that the onset of TdP was delayed by prazosin.

Although ranolazine attenuates methoxamine-induced pressor and reflex bradyarrhythmic effects, just as prazosin does (Fig. 1), the reduction of the methoxamine-induced pressor effect by ranolazine seems to be a physiological (functional) antagonism rather than a pharmacological antagonism. Ranolazine alone at a very high dose (480 μg/kg/min) significantly decreases BP. However, this effect is unlikely to be due to α₁-adrenoceptor antagonism because ranolazine did not cause a significant rightward shift of the phenylephrine dose-response curve. Attenuation of the methoxamine-induced bradyarrhythmia could be one of the potential mechanisms underlying the anti-TdP action of ranolazine because bradyarrhythmia is known to facilitate the occurrence of TdP (Vos et al., 2001; Morissette et al., 2005). However, data from the experiments with lidocaine and prazosin do not support this argument. Lidocaine did not inhibit the bradyarrhythmia caused by methoxamine (Fig. 1) but prevented the occurrence of TdP (Fig. 4). In contrast, prazosin at a dose of 10 μg/kg/min, which slows HR to the same extent as does the highest dose of ranolazine (480 μg/kg/min) (Fig. 1), did not exhibit anti-TdP activity (Fig. 4), suggesting that antagonizing the bradyarrhythmia is not a significant contributor for preventing the occurrence of TdP in this model.

Despite the inhibition of clofilium-induced QT and MAPD prolongation, ranolazine itself prolonged QT and QTc intervals, whereas lidocaine did not (Table 1; Fig. 2). This is due to the fact that ranolazine (but not lidocaine), in addition to inhibition of the late Na current, also blocks I_Kr, with a potency (IC_{50}) of 12 μM (Antzelevitch et al., 2004b; Schram et al., 2004) and causes a prolongation of ventricular repolarization. However, the late I_{Na}-blocking effect of ranolazine counteracts the effect of I_Kr blockade on ventricular repolarization, resulting in only modest (self-limited) APD and QT prolongation. Our results are in agreement with clinical data from MARISA, where ranolazine increased mean QTc interval in a dose-dependent manner, but the increase was less than 10 ms at the highest dose of 1000 mg twice daily (Chaitman et al., 2004). Due to its late I_{Na}-blocking properties, ranolazine, unlike other I_Kr blockers, does not cause TdP both in preclinical (Wu et al., 2004; Antzelevitch and Belardinelli, 2006) or clinical (Chaitman et al., 2004) settings. Consistent with the above findings, the present study using an in vivo model, which has been widely used to investigate the proarrhythmic potential of many I_Kr blockers (Carlsson et al., 1990; Buchanan et al., 1993), demonstrates that at dose range of 120 to 480 μg/kg/min (plasma concentration, ~7–28 μM), ranolazine is not proarrhythmic but instead is antiarrhythmic.


H477–H487.


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