Antiarrhythmic Effects of Ranolazine in a Guinea Pig in Vitro Model of Long-QT Syndrome

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ABSTRACT
Prolongation of the QT interval of the ECG is associated with increased risk of torsades de points ventricular tachycardia. Ranolazine, a novel antianginal agent, is reported to decrease the delayed rectifier potassium current, IKr, and to increase action potential duration (APD) and the QT interval. However, ranolazine is also reported to reduce late sodium current (late INa), a depolarizing current that contributes to prolongation of the plateau of the ventricular action potential. We hypothesized that ranolazine would decrease APD and the occurrence of arrhythmias when late INa is increased. Therefore, we measured the effects of ranolazine alone and in the presence of anemone toxin (ATX)-II, whose action mimics the sodium channelopathy associated with long-QT3 syndrome, on epicardial monophasic action potentials and ECGs recorded from guinea pig isolated hearts. Ranolazine (0.1–50 μM) prolonged monophasic APD at 90% repolarization (MAPD90) by up to 22% but did not cause either early afterdepolarizations (EADs) or ventricular tachycardia (VT), ATX-II (1–20 nM) markedly increased APD and caused EADs and VT. Ranolazine (5–30 μM) significantly attenuated increases in MAPD90 and reduced episodes of EADs and VT produced by ATX-II. Ranolazine also attenuated the synergistic effect of MAPD90 increase caused by combinations of ATX-II and blockers of IKr [E-4031; 1-[2-(6-methyl-2-pyridyl)ethyl]-4-methylsulfonylaminobenzoyl]piperidine]. Thus, although ranolazine alone prolonged APD, it reduced APD and ventricular arrhythmias caused by agents that increased late INa and decreased IKr.

Prolongation of the duration of the ventricular action potential (indicated on the surface electrocardiogram as an increase of the QT interval) due to inhibition of the rapid delayed-rectifier potassium current, IKr, is caused by drugs from many therapeutic classes and is associated with an increased risk of ventricular tachyarrhythmias such as torsades de points (TdP) (Belardinelli et al., 2003). However, QT interval prolongation per se may not be proarrhythmic (Hondeghem et al., 2001). Ranolazine is a novel antianginal drug (Pepine and Wolff, 1999; Louis et al., 2002; Chaitman et al., 2004), and it prolongs the QT interval but is not known to increase the incidence of ventricular tachycardias (VTs) and may reduce the incidence of ischemia-related arrhythmias (Gralinski et al., 1996). At a cellular level, ranolazine has been found to reduce the peak outward current conducted by the delayed rectifier potassium channel IKr with a potency (IC50 value) of 11.5 μM (Zygmunt et al., 2002) and to reduce the late inward sodium current (late INa) with an IC50 of 5 to 21 μM, depending on pacing frequency and membrane potential (Zygmunt et al., 2002; Song et al., 2004).

Inhibitions of IKr and late INa by ranolazine would be expected to have opposite effects on the duration of the QT interval. Whereas inhibition of IKr prolongs action potential duration (APD), inhibition of late INa decreases APD. Thus, the effect of ranolazine on the QT interval may depend on the relative magnitudes of ranolazine-induced changes in IKr and late INa and the contributions of these ion currents to ventricular repolarization. We speculated that ranolazine may be useful to reduce the QT interval and the incidence of early afterdepolarizations (EADs) and TdP when late INa is elevated, as in heritable and/or acquired forms of the long-QT3 (LQT3) syndrome. To test this hypothesis, the electrophysiological effects of ranolazine on the ventricle of the guinea pig isolated heart were determined in the absence and presence of agents that increase late INa and/or decrease repolarizing potassium currents, thereby prolonging the durations of the ventricular action potential and the QT interval. To increase late INa hearts were exposed to Anemone sulcata toxin (ATX)-II. ATX-II prevents full inactivation of the inward sodium current (Isenberg and Ravens, 1984) and therefore
mimics in exaggerated manner mutations of the cardiac sodium channel gene SCN5A that are implicated as the mechanism for the LQT3 form of long-QT syndrome (Bennett et al., 1995; Wang et al., 1995; Priori et al., 1996). Two drugs that are reported to mimic forms 1 and 2 of the long-QT syndrome were used to further prolong the duration of the ventricular action potential in the presence of low concentrations of ATX-II: chromanol 293B (LQT1) and E-4031 (LQT2). Chromanol 293B is a relatively selective blocker of IKs (Sun et al., 2001), the slowly-activating delayed-rectifier potassium current (Barhanin et al., 1996). Mutations in the gene KCNQ1 (KsLQT1) may result in a decrease of IKs, prolongation of action potential duration, and the LQT1 form of long-QT syndrome (Shalaby et al., 1997). E-4031 was shown (Sanguinetti and Jurkiewicz, 1990) to decrease the rapidly activating potassium current IKr, a change that mimics the long-QT syndrome (Shalaby et al., 1997). E-4031 was shown (Sanguinetti and Jurkiewicz, 1990) to decrease the rapidly activating potassium current IKr, a change that mimics the long-QT syndrome (Shalaby et al., 1997). E-4031 was shown (Sanguinetti and Jurkiewicz, 1990) to decrease the rapidly activating potassium current IKr, a change that mimics the long-QT syndrome (Shalaby et al., 1997).

MAP and ECG Measurements. MAPs were recorded using a pressure-contact Ag-AgCl electrode applied to the left ventricular epicardial surface. The electrode was adjusted during an experiment both to maintain stable MAP signals and to prevent damage to the ventricular surface. Electrode signals were amplified (Biopac MP 150, Goleta, CA) and displayed continuously on a computer screen in real time for visual monitoring. In each protocol, to ensure that a response to a given drug concentration had achieved a steady state before the drug concentration was changed, the duration of either MAPD90 or QT interval was measured using an on-screen caliper during the drug infuson period.

To record an ECG, a 1-cm thick sponge ring soaked in saline was placed on the right ventricular free wall. One end of a Teflon-coated tungsten unipolar electrode was inserted in the middle of the sponge and the opposite end plugged into the input of an ECG amplifier. The reference electrode was placed directly into the ventricular epicardial wall close to the A-V valves. ECG, MAP, and coronary perfusion pressure signals were collected in real time and stored on a computer for subsequent analysis. MAP signals were exported to a specially designed Excel template. Signals were used for analysis only when MAPD was stable at pacing rates for at least 10 s and after signal artifacts were removed.

Drug Concentration-Response Relationships. Increasing concentrations of drug were infused in a cumulative manner, allowing 7 to 15 min between changes of concentration. To investigate the rate dependence of effects of drugs on the duration of the ventricular MAP, the ventricular pacing rate was increased stepwise from 1 to 1.5, 2, and 2.5 Hz. Each pacing rate was maintained for 2 min, and MAPs were measured during the last 5 s of each period. The pacing rate was then reduced to 1 Hz, and a 15-min infusion of either ATX-II (7 nM), chromanol 293B (1 μM), or ranolazine (5 μM) was begun. When MAPD90 prolongation attained an apparent steady state in the presence of drugs, the ventricular pacing rate was again increased progressively, and MAPs were recorded.

Determination of Antiarrhythmic Effects of Ranolazine. VT was defined as a sequence of three or more ventricular depolarizations at a rate >1.5 Hz. VT that terminated spontaneously was defined as transient or non sustained VT. VT that did not subside spontaneously unless interrupted by a treatment (ranolazine) was defined as sustained VT. Polymorphic VT (TdP-like VT) was defined as VT with much faster rate and different morphology in both MAP and ECG recordings. A positive depolarization during phase 2 and/or 3 of an antral action potential was defined as an EAD when associated with T wave changes on ECG recording and as a ventricular extrasystolic beat when associated with a QRS complex in the ECG. Confirmation of the spontaneous origin of VT was obtained by its persistence and recurrence when delivery of pacing stimuli was suspended for a minimum of 10 s.

Statistical Analysis. All data are reported as means ± S.E.M. Concentration-response curves were analyzed using Prism version 3.0 (GraphPad Software Inc., San Diego, CA). Repeated measure one-way analysis of variance was used to compare values of measurements obtained from the same heart before and after treatment. When analysis of variance revealed the existence of a significant difference among values, the Student-Newman-Keuls test was applied to determine the significance of a difference between a selected pair of group means. A P value < 0.05 was taken as an upper limit to indicate a significant difference.

Sources of Drugs. Ranolazine [ranexa, (±)-N-(2,6-dimethyl-phenyl)-(4-[2-hydroxy-3-(2-methoxyphenoxy)propyl]-1-piperazine] was synthesized at CV Therapeutics, Inc. CPA were purchased from Sigma-Aldrich (St. Louis, MO), ATX-II and E-4031 (1-[2-(6-methyl-2-pyridyl)ethyl]-4-methylsulfonylaminobenzoyl)-...
piperidine) from Alomone Labs (Jerusalem, Israel), and chromanol 293B (trans-N-[6-cyano-3,4-dihydro-3-hydroxy-2,2-dimethyl-2H-1-benzopyran-4-yl]-N-methyl-ethanesulfonamide) was from Tocris Cookson Inc. (Ellisville, MO).

Results

Effects of ATX-II, Chromanol 293B, E-4031, and Ranolazine on MAPD90, EADs, and Arrhythmias. ATX-II, E-4031, chromanol 293B, and ranolazine each increased the duration of the MAP recorded from the guinea pig heart in a concentration-dependent manner (Fig. 1). E-4031 (10 μM), chromanol 293B (10 μM), and ranolazine (50 μM) increased the duration of the MAP by 50 ± 4% (n = 9), 12 ± 3% (n = 9), and 22 ± 2% (n = 13), respectively (Fig. 1). The maximum effect of ATX-II on MAPD90 could not be determined because ATX-II (>20 nM) caused frequent premature ventricular beats or sustained episodes of VT that interfered with the calculation of MAPD90. High concentrations (1–10 μM) of E-4031 also caused EADs that did not progress to VT in the six hearts studied. Chromanol 293B and ranolazine each caused a moderate prolongation of MAPD90, but did not cause either EADs or VT (Fig. 1).

Attenuation by Ranolazine of the Effect of ATX-II on MAPD90. The concentration-response relationship for ATX-II to increase the duration of the MAP was significantly flattened by 5 μM ranolazine (Fig. 2). Ranolazine (5 μM) alone produced a sustained, small (16% above baseline) prolongation of MAPD90 from 202 ± 9 to 235 ± 4 ms (n = 7), but this prolongation was not additive to that caused by ATX-II. In contrast, the action of ATX-II to lengthen MAPD90 was markedly reduced by ranolazine (Figs. 2 and 3). An example of a record from one of seven hearts exposed to ranolazine in the presence of ATX-II is shown in Fig. 3.

Attenuation by Ranolazine of ATX-II-Induced EADs and VT. Ranolazine greatly reduced the occurrence of EADs, frequent premature ventricular beats, and VT caused by ATX-II. Representative experimental recordings from two of 13 hearts perfused with solution containing 20 nM ATX-II are shown in Fig. 4. Perfusion of hearts with 20 nM ATX-II for 15 min led to significant prolongation of the MAPD and the QT interval, EADs, and ventricular extrasystolic beats, which were followed by episodes of transient and sustained polymorphic VT (Fig. 4, A and B) in all hearts. These rhythm abnormalities were not observed under control conditions in the absence of ATX-II. Administration of 5 to 10 μM ranolazine in the continued presence of 20 nM ATX-II led to the suppression of EADs, abolishment of polymorphic VTs, and restoration of a regular ventricular response to pacing (Fig. 4B, record c). EADs and polymorphic VT reappeared after termination of ranolazine infusion (Fig. 4B, record d). Thus, ranolazine reversibly terminated monomorphic or polymorphic (EAD-triggered) VT caused by ATX-II (20 nM).

Ranolazine not only reversed but also prevented the appearance of EADs, frequent premature ventricular beats, and VT caused by >20 nM ATX-II. VT occurred in all five hearts in Fig. 2 that were treated with 20 nM ATX-II alone. In experiments wherein hearts (n = 7; Fig. 2) were pretreated with 5 μM ranolazine, no EADs or VTs were observed when hearts were subsequently exposed to concentrations of ATX-II as high as 100 nM in the continued presence of ranolazine.

Antagonism by Ranolazine of the MAPD90 Prolongation Caused by ATX-II Plus E-4031 and Chromanol 293B. Ranolazine attenuated the actions of combinations of ATX-II with E-4031 (Fig. 5, A and B) and chromanol 293B (not shown). In hearts paced at 1.5 Hz, perfusion of a low concentration of either ATX-II (7 nM) or E-4031 (1 μM) produced moderate prolongations of MAPD90 (71 ± 8 and 61 ± 9 ms; n = 3 and 4, respectively) (Fig. 5B). Perfusion of ATX-II (7 nM) plus E-4031 (1 μM) produced a marked synergistic increase of MAPD90 by 325 ± 107 ms (Fig. 5B). Ranolazine (5, 10, and 30 μM) significantly attenuated the effects of ATX-II plus E-4031 to prolong MAPD90 (Fig. 5B). Similarly, ranolazine significantly (p < 0.05; n = 4) attenuated the prolongation of MAPD90 caused by a combination of 7 nM ATX-II and 1 μM chromanol 293B. In hearts (n = 4) perfused with chromanol 293B (1 μM) alone or in combina-

Fig. 1. Concentration-response relationships for ATX-II, E-4031, chromanol 293B, and ranolazine to increase duration of the ventricular MAPD90 in guinea pig isolated hearts. Hearts were paced at a rate of 1.5 Hz. Baseline MAPD90 values were 217 ± 5 (n = 5), 212 ± 5 (n = 9), 217 ± 3 (n = 9), and 217 ± 7 ms (n = 13) for ATX-II, E-4031, chromanol 293B, and ranolazine-treated hearts, respectively.

Fig. 2. Attenuation by ranolazine of ATX-II-induced increases of MAPD90. Shown are concentration-response relationships for ATX-II to increase duration of the ventricular MAPD90 in guinea pig isolated hearts, in the absence and presence of 5 μM ranolazine. Hearts were paced at 1.5 Hz. Single and double asterisks indicate that ventricular tachycardia occurred in one and all five hearts studied, respectively.
tion with ATX-II (7 nM), MAPD$_{90}$ was prolonged by 12 ± 4 and 68 ± 3 ms, respectively, above control. In the continued presence of 1 μM chromanol 293B and 7 nM ATX-II, ranolazine (5, 10, and 30 μM) reduced the prolongation of MAPD$_{90}$ from 68 ± 3 to 53 ± 3, 45 ± 3, and 38 ± 5 ms, respectively (n = 4; P < 0.05). Interestingly, ranolazine neither increased nor decreased MAPD$_{90}$ in the presence of E-4031 alone (i.e., in the absence of ATX-II). E-4031 (1 μM) increased MAPD$_{90}$ by 54 ± 9 ms from 211 ± 9 (control) to 265 ± 9 ms (n = 5; P < 0.05). Values of MAPD$_{90}$ in the presence of 1 μM E-4031 with 5, 10, and 30 μM ranolazine were 267 ± 10, 266 ± 11, and 263 ± 14 ms, respectively (n = 5; P > 0.05 versus E-4031 alone). In contrast, ranolazine increased MAPD$_{90}$ in the presence of the I$_{Ks}$ blocker chromanol 293B. Chromanol 293B (1 μM) increased MAPD$_{90}$ by 14 ± 6 (n = 5) ms above control.

Values of MAPD$_{90}$ in the presence of chromanol 293B alone and with 5, 10, and 30 μM ranolazine were 208 ± 4, 220 ± 5, 224 ± 6, and 240 ± 6 ms, respectively (n = 5; P < 0.01 for 30 μM ranolazine versus chromanol 293B alone).

**Lack of Rate Dependence of Action of Ranolazine on Duration of the MAP.** Antiarrhythmic drugs must be effective at high heart rates; therefore, knowledge of the dependence of drug action on heart rate is useful to predict efficacy of a prospective antiarrhythmic agent (Hondeghem and Snyders, 1990). Reverse use dependence is considered to be a proarrhythmic risk factor. The effect of a decrease in pacing cycle length (increase of pacing frequency) on prolongations of MAPD$_{90}$ by ranolazine, ATX-II, E-4031, and chromanol 293B is shown in Fig. 6. As expected, duration of the MAP decreased with a decrease in pacing cycle length in control
hearts (Fig. 6, left). Ranolazine and chromanol 293B did not alter this relationship, whereas E-4031 and ATX-II steepened it (Fig. 6, left). This is shown more clearly in Fig. 6, right, where the differences in values of MAPD\(_{90}\) between drug-treated and control hearts are plotted as a function of pacing cycle length. An absence of rate dependence is indicated by a zero slope of the relationship between cycle length and MAPD\(_{90}\) (i.e., a line parallel to the abscissa). Prolongations of MAPD\(_{90}\) caused either by ranolazine or by the I\(_Ks\) blocker chromanol 293B were independent of rate, with slope values that were not significantly different from zero. On the other hand, the frequency-response plots describing the effects of E-4031 and ATX-II on MAPD\(_{90}\) had significantly positive slopes (0.018 ± 0.003 and 0.026 ± 0.07, respectively), indicative of a reverse rate dependence of the effects of E-4031 and ATX-II.

**Discussion**

Drug-induced reduction of I\(_{Kr}\), prolongation of the QT interval, and TdP are a potential risk during treatment with a wide range of drugs from diverse therapeutic classes (Hondeghem et al., 2001; Belardinelli et al., 2003). Therefore, we sought to determine whether the APD prolongation caused by ranolazine was associated with proarrhythmic activity. In keeping with its effect to reduce outward potassium current (I\(_{Kr}\) blockade), ranolazine prolonged left ventricular MAPD\(_{90}\). However, as now acknowledged by many investigators, some drugs that prolong the duration of the ventricular action potential and the QT interval do not increase the risk of TdP (Hondeghem et al., 2001; Studenik et al., 2001; van Opstal et al., 2001). Thus, of particular interest are the results that 1) ranolazine alone did not cause EADs, ectopic beats, and VT; 2) ranolazine markedly reduced the proarrhythmic effects of ATX-II (Figs. 2–4); and 3) ranolazine reduced the duration of the MAP in the presence of a combination of two agents (ATX-II and E-4031) that act by different mechanisms to increase MAPD\(_{90}\) (Fig. 5). The mechanism by which ranolazine decreased APD in the presence of ATX-II and 1 \(\mu\)M E-4031 (Fig. 5) is likely to be a reduction of late I\(_{Na}\). Because ranolazine decreased ventricular MAPD in the presence of combinations of ATX-II with either E-4031 or chromanol 293B, but not in the presence of the I\(_{Kr}\) blockers alone (i.e., absence of ATX-II), the data suggest that ranolazine is effective to reduce MAPD only when late I\(_{Na}\) contributes significantly to prolongation of the action potential. However, it is also possible that ranolazine and E-4031 compete for the same binding site in the HERG channel, because both ranolazine and E-4031 inhibit I\(_{Kr}\). The result that ranolazine markedly reduced prolongation of the MAPD caused by ATX-II supports the findings by Zygmunt et al. (2002) and Song et al. (2004) that ranolazine decreased late I\(_{Na}\). ATX-II is reported to inhibit inactivation of the cardiac Na\(^+\) channel,
thus increasing both the inward current entering the cardiomyocyte during the plateau of the action potential, and action potential duration. This “gain of function” by the sodium channel delays repolarization and facilitates the development of EADs (Isenberg and Ravens, 1984; Studenik et al., 2001). The actions of ATX-II resemble those caused by mutations in the gene SCN5A (Bennett et al., 1995; Wang et al., 1995) that lead to repeated openings of sodium channels during the action potential plateau. Thus, the guinea pig isolated heart exposed to ATX-II is a model for the human LQT3 syndrome, which is characterized by a long QT interval and increased susceptibility to TdP induced by drugs that reduce outward currents during the action potential plateau, namely, blockers of IK (Makita et al., 2002). Patients with LQT3 syndrome are at increased risk of TdP (Rodên, 2000; Schwartz et al., 2001). In hearts pretreated with ATX-II, ranolazine not only did not further prolong, but in contrast, shortened the duration of the MAP. Thus, it seems that the action of ranolazine to inhibit late INa has more effect on action potential duration than its action to block IKr under conditions that cause an increase of late INa.

A recent report of the effects of ranolazine to attenuate ATX-II-induced increases of late INa and EADs in guinea pig isolated ventricular myocytes (Song et al., 2004) is consistent with the results shown in the present study. Thus, it is likely that the afterdepolarizations caused by ATX-II (and abolished by ranolazine) noted in recordings of MAP in this study (Fig. 4A) are indicative of the presence of EADs, as we have concluded. The effects of ranolazine to reduce late INa, ventricular arrhythmias and prolongation of the MAP in the presence of ATX-II are similar to those of mexiletine, a class IB antiarrhythmic agent (sodium channel blocker) (Shimizu and Antzelevitch, 1997). Mexiletine was found to have a greater effect to block SCN5A mutant sodium channels than to block wild-type sodium channels (Wang et al., 1997).

Increased late INa and reductions in repolarizing K+ currents, whether caused by drugs or by heritable ion channel dysfunction, prolong the QT interval, and are major predisposing factors for TdP in humans (Sasyiniuk et al., 1989). Prolongation of the QT interval beyond a certain limit may herald proarrhythmic events (Shaffer et al., 2002). If life-threatening ventricular tachycardia episodes are almost invariably the consequence of a considerably prolonged QT interval, then a drug like ranolazine with the property of limiting or reducing drug or disease-induced prolongation of the QT interval may be of therapeutic value. However, much additional research is required before the present results can be extrapolated to clinical practice. The temporal and spatial patterns of electrical activity, ion channel expression, and physiological/pathological regulation of ion channel function of the human heart ventricle are not fully replicated in hearts of animals used for experimentation. In particular, as regards the present study, the potassium currents of guinea pig ventricular myocytes differ in relative magnitude from those of human ventricular myocytes; the magnitude of IK, is less, whereas the magnitude of IKr is greater in the guinea pig compared with the human ventricular myocyte (Zicha et al., 2003).

In contrast to classical class III antiarrhythmic agents and E-4031, which block IKr, ranolazine prolonged cardiac repolarization in a way that did not wane when pacing rate was increased. Ideally, ventricular tachycardias would be treated with an antiarrhythmic agent whose effectiveness rose as heart rate increased (Hondeghe and Snyder, 1990). Unfortunately, many class III antiarrhythmic agents are less effective in prolonging action potential duration at high than at low heart rates (Hondeghe and Snyder, 1990). In conclusion, the results of this investigation indicate that the prolongation of action potential duration by ranolazine seems not to be associated with an increased probability of ventricular arrhythmias (EADs or VT). This finding lends credence to the proposal that prolongation of action potential duration per se is not proarrhythmic. Ranolazine reduced the prolongation of cardiac repolarization caused by drugs that mimic ion channelopathies associated with increased late INa or decreased IK and terminated episodes of ATX-II-induced nonsustained and sustained ventricular tachycardia. The prolongation of MAPD caused by ranolazine was independent of ventricular rate. Thus, under circumstances where late INa is increased, ranolazine seems to act as an antiarrhythmic drug.

References


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