Abstract

Assessment of the proarrhythmic risk associated with drugs that prolong the QT interval is difficult. We hypothesized that the proarrhythmic activities of drugs with very low to moderate risk of causing torsades de pointes would be well differentiated when the late sodium current (I_{NaL}) was greater than normal. The effects of selected QT-prolonging drugs on electrical activity of female rabbit isolated hearts were determined in the absence and presence of sea anemone toxin (ATX-II; an enhancer of I_{NaL}). I_{NaL} recorded from ventricular myocytes isolated from female rabbit hearts was slightly increased by 1 and 3 nM ATX-II (n = 13, P < 0.01). ATX-II (1 nM) prolonged the duration of the monophasic action potential (MAPD) of the isolated heart by 19 ± 3% (P < 0.001, n = 31) and shifted the concentration-response relationships for cisapride (1–30 nM), ziprasidone (0.01–3 μM), quinidine (0.1–1 μM), and moxifloxacin (0.01–1 μM) to prolong MAPD_{90} to the left by 2- to 12-fold. In contrast, the increases in MAPD_{90} caused by 1 nM ATX-II and pentobarbital were only additive, and the increases in MAPD_{90} caused by ATX-II and ranolazine [(±)-N-(2,6-dimethylphenyl)-(4-[2-hydroxy-3-(2-methoxyphenoxy)]propyl)-1-piperazine] were less than additive. Episodes of arrhythmic activity were commonly observed, and beat-to-beat variability of action potential duration was increased, during exposure of hearts to cisapride, ziprasidone, quinidine, and moxifloxacin but not during exposure of hearts to ranolazine or pentobarbital, in the presence of ATX-II. Thus, in the female rabbit heart, ATX-II potentiated the effects of QT-prolonging drugs to increase MAPD_{90} and unmasked the proarrhythmic activities of these drugs at clinically relevant drug concentrations.

ABBRéviations: TdP, torsades de pointes; ECG, electrocardiogram; VT, ventricular tachycardia; ATX-II, sea anemone toxin; ranolazine, (±)-N-(2,6-dimethylphenyl)-(4-[2-hydroxy-3-(2-methoxyphenoxy)]propyl)-1-piperazine; K-H, Krebs-Henseleit; CPP, coronary perfusion pressure; MAP, monophasic action potential; MAPD, monophasic action potential duration; BVR, beat-to-beat variability of repolarization of MAPD_{90}; EVB, ectopic ventricular beat; EAD, early after depolarization; LV, left ventricular.
SCN5A mutations or to organic heart disease (e.g., heart failure) is also associated with a decrease of repolarization reserve and an increased susceptibility to TdP (Bennett et al., 1995; Makita et al., 2002; Splatowski et al., 2002). Furthermore, an increase of INaL causes increases of intracellular sodium content and sodium extrusion/calcium entry via the sodium-calcium exchanger and may lead to intracellular calcium overload. Therefore, we hypothesized that a small augmentation of INaL would potentiate and unmask the proarhythmic effects of QT-prolonging drugs, including drugs that have a very low risk of causing ventricular tachycardia (VT). The methodology of this study was to evaluate the facilitation by ATX-II (an enhancer of INaL) of the proarhythmic effects of QT-prolonging drugs in the female rabbit isolated, perfused heart. The female rabbit heart was chosen for its high sensitivity to human ether-a-go-go-related gene (i.e., 1NaK channel blockade and its relatively low repolarization reserve (Joshi et al., 2004).

The drugs selected for evaluation in this study were cisapride, quinidine, ziprasidone, moxifloxacin, pentobarbital, and ranolazine. These drugs have different pharmacological activities but share an effect to inhibit IKr. The risk of proarhythmic activity associated with the clinical use of each drug, however, is quite different. Cisapride (Propulsid), a motility enhancer that can be used to treat patients with gastroesophageal reflux disease, and quinidine, a class Ia antiarrhythmic agent, have been shown to prolong the QT interval and to trigger TdP (Roden et al., 1986; Wysowski and Anderson, 2002). Pentobarbital, a barbiturate, and ranolazine, an antianginal agent, have been shown to prolong the QT interval without causing TdP in cardiac preparations from laboratory animals or in clinical use (Shimizu et al., 1999; Song et al., 2004; Wu et al., 2004). Therefore, in this study, we consider cisapride and quinidine as drugs with a very low risk for causing TdP.

### Materials and Methods

#### Animal Model.
This investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (National Institutes of Health publication 85-23, revised 1996). Animal use for this project was approved by the Institutional Animal Care and Use Committee of CV Therapeutics (Palo Alto, CA). New Zealand White female rabbits, weighing 2.5 to 3.5 kg, were sedated then anesthetized using i.m. and i.v. injections, respectively, of xylazine and ketamine. The thorax was opened, and the heart was excised and placed in a modified Krebs-Henseleit (K-H) solution (pH 7.4, gassed with 95% O2 and 5% CO2). The K-H solution contained 118 mmol/l NaCl, 2.8 mmol/l KCl, 1.2 mmol/l KHPO4, 2.5 mmol/l CaCl2, 0.5 mmol/l MgSO4, 2.0 mmol/l pyruvate, 5.5 mmol/l glucose, 0.57 mmol/l Na2EDTA, and 25 mmol/l NaHCO3. The aorta was cannulated, and the heart was perfused by the method of Langendorff with K-H solution warmed to 36.5°C at a rate of 20 ml/min. Coronary perfusion pressure (CPP) was measured from a side port of the aortic cannula. The right atrial wall was partially removed. Complete atrioventricular block was induced by thermoablation of the atrioventricular nodal area. A bipolar Teflon-coated electrode was placed on the right ventricular septum to pace the heart. Electrical stimuli, 3 ms in width and 3-fold threshold amplitude, were delivered to the pacing electrode at a frequency of 1 Hz using a Grass Instruments (Quincy, MA) S48 stimulator. After initiation of ventricular pacing, a 30- to 50-min period was allowed for equilibration of heart rhythm and CPP. The total duration of the experimental protocol was ≥2.5 h.

#### Monophasic Action Potential (MAP) and ECG Recording.
MAP and ECG electrodes were used to record left ventricular MAPs and bipolar ECGs, respectively. Pressure-contact Ag-AgCl MAP electrodes were placed on the epicardial ventricular free wall below the level of the atrial-ventricular valve at the base of the left ventricle. The duration of the MAP at 100% repolarization was observed and monitored with an on-screen caliper. MAP profiles were analyzed by computer to determine the duration of the MAP at the level at which repolarization is 90% completed (MAPD90). MAPs, ECGs, and CPP signals were digitized in real time and displayed on a computer monitor. Steady-state responses to drug(s) are reported in this study.

#### Beat-to-Beat Variability of MAPD90.
Values of MAPD90 for 30 consecutive beats were used for calculation of the beat-to-beat variability of ventricular repolarization (BVR). As a measure of BVR, the mean orthogonal distance on the Poincaré plot from the diagonal to each point was determined using the following equation:

$$\Sigma |\text{MAPD}_{n-1} - \text{MAPD}_n| / (30 \times v^2)$$

(Thomsen et al., 2004).

#### Measurements of Transmembrane Potential and 1NaK from Rabbit Ventricular Myocytes.
To measure the effect of ATX-II on myocyte INaL, single myocytes were isolated by collagenase digestion of rabbit hearts as described by Song et al. (2004) for guinea pig hearts with the following modifications: hearts were perfused at a rate of 16 ml/min; the activity of collagenase used for digestion was 240 U/ml; and the volume of collagenase-containing solution was 70 ml, and it was recirculated through the heart for a period of 40 min. Transmembrane voltages and currents were recorded from quiescent myocytes using borosilicate glass capillary microelectrodes (1–3 MΩ resistance when filled) in a whole-cell patch-clamp configuration. An Axopatch-200 amplifier, a DigiData-1200A interface, and a computer with pCLAMP8 software (Molecular Devices, Sunnyvale, CA) were used to amplify, store, and analyze the recorded signals. For recording of late INaL, K+ and Ca2+ were omitted from both the bath Tyrode’s solution and the microelectrode solution to reduce contamination of IS by K+ and Ca2+ currents. Recording microelectrodes were filled with 120 mmol/l Cs-aspartate, 20 mmol/l CsCl, 1 mmol/l MgSO4, 4 mmol/l Na2ATP, 0.1 mmol/l Na4GTP, 1 mmol/l EGTA, and 10 mmol/l HEPES, pH 7.2. Myocytes were voltage-clamped at a holding potential of −90 mV. The electrode capacitance, whole-cell capacitance, and series resistance were maximally compensated. The liquid junction potential was measured and nulling used the pCLAMP program. To elicit INaL, a 300-ms depolarizing pulse to −20 mV was applied at a frequency of 0.16 Hz. The magnitude of late INaL was determined by integration of the area (nA × ms = nC) of the current over the last 50 ms of the −20-mV clamp pulse, using the integration (area) feature of the pCLAMP program.
Determination of Concentration-Response Relationships for Effects of Drugs in the Absence and Presence of 1 nM ATX-II in Rabbit Isolated, Perfused (Method of Langendorff) Hearts. Rabbit hearts were exposed to increasing concentrations of cisapride (10–600 nM), quinidine (0.1–30 μM), ziprasidone (0.1–10 μM), and moxifloxacin (0.1–100 μM) in a cumulative manner, allowing 7 to 15 min between increases in drug concentration to facilitate the recording of a steady-state, maximal effect. All hearts were paced at a rate of 1 Hz throughout the experimental procedure. Drug effects (concentration-response relationships) were measured in the absence and presence of ATX-II. After recording of drug responses in the absence of ATX-II, a heart was perfused with K-H solution containing 1 nM ATX-II for 20 min, and drug administration was repeated in the continued presence of ATX-II.

Determination of Proarrhythmic and Antiarrhythmic Activities of QT-Prolonging Drugs in the Absence and Presence of ATX-II. Ventricular arrhythmic activity, including ectopic ventricular beats (EVBs), early after-depolarizations (EADs), and VT, was monitored continuously during drug treatment of the isolated heart. Postdrug control values of MAPD were obtained when drug washout was completed. An EVB was defined as a spontaneous beat occurring earlier than the next paced beat. VT was defined as a sequence of three or more consecutive spontaneous ventricular depolarizations at a rate exceeding the pacing rate. An EAD was defined as the positive depolarization during phase 2 and/or 3 of an action potential. 

Statistical Analysis. Data are reported as means ± S.E.M. Concentration-response curves were analyzed using Prism Version 3.0 (GraphPad Software Inc., San Diego, CA). To compare values of measurements obtained from the same heart before and after a drug treatment, repeated measures one-way analysis of variance was used, and Student-Newman-Keuls test was applied to determine the significance of the difference between two means before (as control) and after drug treatment in same or different hearts, respectively. A significant difference (concentration-response relationships) was measured in the absence and presence of ATX-II. To compare values of MAPD90 in rabbit hearts was 163 to 212 ms (mean, 184 ± 2 ms; n = 78), and the range of values of MAPD90 in the presence of 1 nM ATX-II alone was 177 to 305 ms (mean, 218 ± 4 ms; n = 31). Cisapride (10–600 nM, n = 6), ziprasidone (0.1–10 μM, n = 6), quinidine (0.1–30 μM, n = 6), and moxifloxacin (0.1–100 μM, n = 7) each significantly (P < 0.01) prolonged the duration of MAPD90 in a concentration-dependent manner (Fig. 2). The maximal increases of MAPD90 (recorded before the occurrence of frequent ventricular arrhythmias precluded the measurement of MAPD) caused by cisapride, ziprasidone, quinidine, and moxifloxacin were 32 ± 5%, 21 ± 5%, 43 ± 7%, and 37 ± 7%, respectively [P < 0.01 for the difference between control (no drug) and each drug treatment; Fig. 2]. ATX-II (1 nM) enhanced the effects of cisapride, ziprasidone, quinidine, and moxifloxacin on MAPD90 (Fig. 2). ATX-II alone prolonged MAPD90 by 19 ± 3% from 184 ± 2 to 218 ± 4 ms (P < 0.001, n = 31) without causing ventricular tachycardia (data not shown). In the presence of 1 nM ATX-II, the concentration-response relationships for cisapride (1–30 nM, n = 6, Fig. 2A), ziprasidone (0.01–3 μM, n = 7, Fig. 2B), quinidine (0.1–3 μM, n = 7, Fig. 2C), and moxifloxacin (0.1–30 μM, n = 6, Fig. 2D) to prolong MAPD90 were shifted to the left. The estimated relative potencies (EC50 values) for cisapride, ziprasidone, quinidine, and moxifloxacin to prolong MAPD90 were increased in the presence of ATX-II by 12-, 2-, 5-, and 5-fold, respectively (Fig. 2).
Ranolazine (0.1–100 μM, n = 7) prolonged MAPD₉₀ by up to 52 ± 6 ms (Fig. 3A, P < 0.001). However, when hearts were pretreated with 1 nM ATX-II, the prolongation of MAPD₉₀ by ranolazine was only 31 ± 6 ms (n = 6, P < 0.05), and when hearts were pretreated with 2 nM ATX-II, MAPD₉₀ was decreased by ranolazine (Fig. 3A). Pentobarbital (10–300 μM, n = 7) prolonged MAPD₉₀ by 21 ± 5 ms from 185 ± 6 to 205 ± 8 ms (P < 0.001; Fig. 3B). The increases of MAPD₉₀ caused by 1 nM ATX-II and by pentobarbital were additive (Fig. 3B).

**Proarrhythmic Effects of Cisapride, Quinidine, Ziprasidone, and Moxifloxacin in the Absence and Presence of 1 nM ATX-II.** Cisapride, quinidine, ziprasidone, and moxifloxacin caused arrhythmic activity (VT) in female iso-
lated rabbit hearts (Figs. 2, 4, 5, and 6). In the absence of ATX-II, cisapride (600 nM, n = 5) caused VT in all hearts (Fig. 2). Cisapride (≤300 nM) caused VT in only four of six hearts. In the presence of 1 nM ATX-II, however, the effective concentration of cisapride to cause VT was reduced such that 30 nM cisapride caused EVBs, EADs, and VT in six of six hearts (Fig. 2). Quinidine (1 μM) alone caused EVBs and nonsustained VT in one of six hearts. In contrast, in the presence of 1 nM ATX-II, quinidine (1 μM) caused EVBs, EADs, and VT in seven of seven hearts (Fig. 2). The effects of ziprasidone and moxifloxacin to cause VT were also enhanced in the presence of 1 nM ATX-II (Fig. 2). In contrast, ranolazine (0.1–100 μM) and pentobarbital (10–300 μM) both prolonged MAPD_{90} but caused no arrhythmic activity in either the absence or presence of ATX-II (Fig. 3). The concentrations of ziprasidone to induce VT were approximately 10-fold lower in the presence than in the absence of ATX-II (Figs. 4 and 5). Moxifloxacin at a concentration of 1 μM did not cause either VT or pause-triggered arrhythmic activity (Figs. 6 and 7). When administered at concentrations as high as 60 to 100 μM (Figs. 2D and 6B), moxifloxacin caused VT in only three of eight hearts. However, in the presence of 1 to 3 nM ATX-II, moxifloxacin (1–3 μM) prolonged MAPD_{90} and caused VT in 12 of 13 hearts studied (Figs. 2D and 6D). ATX-II (3 nM) alone prolonged MAPD_{90} by 28 ± 8% from 177 ± 5 to 225 ± 15 ms but caused neither EADs nor VT (Fig. 6C). ATX-II (3 nM) and moxifloxacin (1 μM) together caused spontaneous and paused-triggered EADs, EVBs, and polymorphic VT in seven of seven hearts studied (Figs. 6D and 7C).

Suppression by Ranolazine of Spontaneous or Pause-Triggered Ventricular Arrhythmic Activity in the Presence of Proarrhythmic Agents. Ranolazine terminated arrhythmic activity caused by 1 μM moxifloxacin in the presence of 3 nM ATX-II (Fig. 6E). ATX-II (3 nM) and moxifloxacin (1 μM) together caused spontaneous EADs, EVBs, and VT (Fig. 6D). Ranolazine (5, 10, and 30 μM) shortened MAPD_{90} and abolished VT in four, five, and seven, respectively, of seven hearts. VT reappeared within 10 to 15 min after discontinuation of ranolazine treatment in six of seven hearts (Fig. 6F) in the continued presence of ATX-II (3 nM) and moxifloxacin (1 μM). In the presence of 1 μM moxifloxacin, a 3-s pause caused neither EADs nor ventricular arrhythmic activity (n = 5, Fig. 7B). However, in the presence of both 3 nM ATX-II and 1 μM moxifloxacin, a 3-s pause caused EADs, EVBs, and/or nonsustained VT (Fig. 7C). Ranolazine (5–30 μM) abolished the EADs, EVBs, and VT triggered by 3-s pauses in the presence of ATX-II (3 nM) + moxifloxacin (1 μM) (n = 5). Ranolazine also suppressed the spontaneous and pause-triggered arrhythmic activity caused by cisapride, quinidine, and ziprasidone in the presence of 1 nM ATX-II (data not shown).

**BVR of Ventricular Repolarization Caused by Drugs in the Absence and Presence of ATX-II.** Short-term BVR was measured during a control (no drug) period, in the presence of ATX-II or drug alone, and in the presence of drug + 1 nM ATX-II (Fig. 8). ATX-II (1 and 3 nM; n = 26 and 6, respectively), quinidine (1 μM; n = 6), moxifloxacin (1 μM; n = 6), and ziprasidone (1 μM; n = 6) significantly increased the BVR (P < 0.05 and 0.01, respectively). The increases of BVR caused by quinidine (1 μM), moxifloxacin (1 μM), and ziprasidone (1 μM) were significantly greater (P < 0.01) in the presence than in the absence of 1 nM ATX-II (Fig. 8). For example, cisapride (Cisa, 30 nM) alone did not increase BVR (n = 5, P > 0.05 compared with control; Fig. 8A) but significantly increased BVR in the presence of ATX-II (n = 6, P < 0.01, Fig. 8B) and caused TdP. Although both ranolazine (0.1–100 μM) and pentobarbital (10–300 μM) prolonged MAPD_{90}, neither drug increased BVR in either the absence (Fig. 8A) or presence (Fig. 8B) of 1 nM ATX-II.

**Discussion**

Drug-induced QT prolongation is most commonly associated with drugs that inhibit the rapid component of the delayed rectifier potassium current, Ik_{r}. The use of drugs that prolong the QT interval is considered to increase the risk of TdP in humans (Haverkamp et al., 2000; Haddad and Anderson, 2002; Belardinelli et al., 2003; Joshi et al., 2004). However, the incidence of TdP caused by drugs that prolong the QT interval does not correlate with the extent of QT prolongation by the same drugs (Haverkamp et al., 2000; Belardinelli et al., 2003). Thus, QT interval prolongation is not an adequate indicator of the drug-induced risk of TdP, and the rare occurrence of TdP makes the direct assessment of risk difficult. The size of clinical trials is insufficient to detect a very rare incident of TdP.

The present study shows that the female rabbit heart exposed to ATX-II is a very sensitive model for detection of proarrhythmic effects of QT-prolonging drugs. ATX-II increases INaL, and thereby decreases the net repolarizing current. This model can differentiate QT-prolonging drugs with proarrhythmic activity and the total number of hearts studied.

![Fig. 4](image-url) Proarrhythmic effect of ziprasidone in the absence (open bars, n = 5) and presence (filled bars, n = 11) of 1 nM ATX-II. The proarrhythmic effect of ziprasidone was potentiated by ATX-II. Values in parentheses represent the cumulative number of hearts with ventricular arrhythmic activity and the total number of hearts studied.
to 10.2, 0.18 to 0.59, and 1.3 to 10.7 μM, respectively (Hatlebak and Berstad, 1996; Stass et al., 1998; Campbell and Williams, 2001; Miceli et al., 2005). At these respective concentrations, cisapride, quinidine, and moxifloxacin caused significant MAPD prolongation, EADs, frequent ventricular premature beats, and polymorphic VTs in the rabbit heart exposed to 1 nM ATX-II. Ziprasidone caused VT only at concentrations (i.e., ≥0.3 μM) that are equal to or exceed the therapeutic range of drug concentrations. Therapeutic concentrations of ranolazine and pentobarbital are 1 to 10 and 4 to 20 μM, respectively (Hart AP et al., 1997; Chaitman et al., 2004b). At these (and higher) concentrations, neither drug caused arrhythmic activity in either the absence or presence of ATX-II.

The finding that ATX-II increases proarrhythmic actions of cisapride, quinidine, moxifloxacin, and ziprasidone suggests that these drugs may be unsafe in patients in which late \( \text{INa} \) is increased due to heritable (i.e., SCN5A) or acquired channelopathies. \( \text{INa,L} \) is reported to be increased in myocytes from both canine and human failing hearts, in myocytes exposed to oxygen free radicals, in postmyocardial infarction “remodeled” myocytes, and in hypoxic and ischemic hearts (Ward and Giles, 1997; Undrovinas et al., 2002; Valdivia et al., 2005). The increase in \( \text{INa,L} \) causes a decrease in repolarization reserve and may cause cytosolic calcium overload. Reduced repolarization reserve has been used to explain the increased proarrhythmic risk of QT-prolonging drugs in animal and human hearts (Roden, 1998). An increase of \( \text{INa,L} \) and a decrease of \( \text{IKr} \) both reduce repolarization reserve. Thus, the effects of cisapride, moxifloxacin, and ziprasidone to increase MAPD in the presence of ATX-II in the present study can be explained as the result of two actions, both of which decrease repolarization reserve. Our results are consistent with the finding (Song et al., 2004) that ATX-II and blockers of \( \text{IK} \) have synergistic actions to increase the duration of the action potential of guinea pig ventricular myo-
cytes. Our results are also consistent with the report that veratridine, an inhibitor of sodium channel inactivation, caused EADs, TdP, and increased transmural dispersion of repolarization in Langendorff-perfused rabbit hearts (Milberg et al., 2005). Although the increase of late INa caused by ATX-II may not accurately mimic the many other conditions that predispose to drug-induced VT, inasmuch as the effect of these conditions to cause VT depends on a reduction of repolarization reserve, that effect may be reproduced by exposure of the heart to ATX-II.

An increase of BVR has also been used to predict proarrhythmic activity of QT-prolonging drugs (Hondeghem and Hoffmann, 2003; Thomsen et al., 2004). The results of the present study indicate that the changes in BVR caused by drugs in the absence and presence of an INaL enhancer are consistent with the effects of the same drugs to cause VT in the presence of ATX-II.

Ranolazine and pentobarbital were found in this study to prolong the MAPD, consistent with previous reports (Wu et al., 2004). Ranolazine did not cause arrhythmic activity in either the absence or presence of ATX-II, and in the presence of 2 nM ATX-II, ranolazine actually decreased MAPD90. Pentobarbital did not cause arrhythmic activity in the absence or presence of ATX-II, but its effect and that of ATX-II to in-

Fig. 7. Inhibition by ranolazine of pause-triggered EADs and ventricular arrhythmias in the presence of ATX-II + moxifloxacin in a female rabbit isolated heart paced at 1 Hz. MAPs (top record in each panel) and ECGs (bottom record) were recorded sequentially before and after a 3-s pause in the absence of drug (A) and in the presence of moxifloxacin alone (B), ATX-II + moxifloxacin (C), ATX-II + moxifloxacin + ranolazine (D), and after washout of ranolazine (E). Arrows, EADs, extraventricular beats, and ventricular tachycardia.

Fig. 8. Effects of QT-prolonging drugs on BVR in the absence (A) and presence (B) of 1 nM ATX-II in female rabbit isolated hearts. The value of BVR in the absence of drug (control; A) was 0.43 ± 0.02 ms. Numbers in parentheses represent the number of experiments. Asterisks indicate the statistical significance of a difference from control in A or from ATX-II (1 nM) alone in B; *, P < 0.05; **, P < 0.01.
crease MAPD\textsubscript{90} were additive. Neither ranolazine nor pentobarbital increased BVR in the absence or presence of ATX-II. In contrast, cisapride, quinidine, moxifloxacin, and ziprasidone each increased BVR in the presence of 1 nM ATX-II. An increase of BVR was reported to predict drug-induced TdP in dog (Thomsen et al., 2005).

Our results with both pentobarbital and ranolazine confirm the opinion that QT prolongation per se is not a good predictor of TdP because both drugs prolong the QT interval but are not reported to cause TdP (Shimizu et al., 1999; Zhou et al., 2002; Chaitman et al., 2004a,b). Furthermore, equal prolongations of MAPD\textsubscript{90} in the present study were not associated with equal propensities for arrhythmic activity. Prolongations of MAPD\textsubscript{90} of 60 ms caused by either quinidine or moxifloxacin in the absence of ATX-II were not associated with VT, whereas 60-ms prolongations of MAPD\textsubscript{90} caused by either drug in the presence of ATX-II were associated with the occurrence of VT in all hearts (Fig. 2). The mechanisms underlying the proarrhythmic activities of drugs include increases of MAPD, transmural dispersion of repolarization, beat-to-beat variability of action potential duration, triangulation, and induction of EADs (Belardini et al., 2003; Hondeghem et al., 2003; Antzelevitch et al., 2004; Thomsen et al., 2005). For the pure IKr blockers, cisapride, moxifloxacin, and ziprasidone, drug effects to increase MAPD prolongation and to induce arrhythmic activity were synergistically increased by ATX-II. However, the effects of quinidine, ranolazine, and pentobarbital to increase MAPD and to cause arrhythmic activity were not directly related, and the effects of ranolazine and pentobarbital were not potentiated by ATX-II. Quinidine, ranolazine, and pentobarbital are “blockers” of more than one ion current. Ranolazine inhibits both IKr and INa\textsubscript{sat}. Reductions of IKr and INa\textsubscript{sat} have opposite actions on action potential duration. In the absence of ATX-II, the inhibition of IKr predominated and ranolazine increased MAPD\textsubscript{90}; in the presence of 2 nM ATX-II, the inhibition of INa\textsubscript{sat} by ranolazine predominated over its action to inhibit IKr, and ranolazine shortened MAPD. This may explain the findings that ranolazine did not cause arrhythmias in either the presence or absence of ATX-II and reversed the ventricular arrhythmic activity caused by moxifloxacin, quinidine, cisapride, and ziprasidone (this manuscript; Song et al., 2004; Wu et al., 2004).

In conclusion, the results of assays of effects of six QT-prolonging drugs on MAPD\textsubscript{90} and arrhythmic activity in the female rabbit isolated, perfused heart exposed to a low concentration of ATX-II (an enhancer of late INa) appear to correlate with the known risks for each drug to cause TdP in patients. Therefore, this preparation can be useful in preclinical studies to predict the risk that a drug candidate will cause TdP when late INa is increased. Assays with this preparation are able to detect the proarrhythmic potential of drugs that are known to have a very low proclivity to cause TdP.

References


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