Augmentation of Late Sodium Current Unmasks the
Proarrhythmic Effects of Amiodarone

Wu et al: Pro- and Anti-arrhythmic Effects of Amiodarone

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ABSTRACT

Aim: Clinical use of amiodarone is associated with occasional development of torsade de pointes (TdP). However, preclinical models have failed to demonstrate the proarrhythmic potential of amiodarone. The objective of this study was to reveal and explain the pro- and anti-arrhythmic effects of acute exposure to amiodarone in an animal model. Methods: Endo- and epicardial monophasic action potentials (MAPs) and 12-lead ECG were recorded in female rabbit isolated hearts. Ion channel currents were measured in human embryonic kidney cells expressing SCN5A Na$^+$ and HERG K$^+$ channels. Results: Acute amiodarone alone caused an insignificant increase in duration of MAP (MAPD$_{90}$) without causing TdP. In the presence of 3 nM sea anemone toxin (ATX-II), amiodarone (1-30 nM) prolonged MAPD$_{90}$ from 217±5 to 250±8 ms (n=16, p<0.01), increased transmural dispersion of repolarization (TDR) from 59±9 to 70±10 ms and beat-to-beat variability (BVR) of MAPD$_{90}$ from 0.75±0.03 to 1.06±0.13 ms (p<0.05). At 30-300 nM, amiodarone induced TdP in 16 out of 17 hearts. A further increase of amiodarone concentration to 1-10 μM abbreviated MAPD$_{90}$ to 211±9 ms, decreased BVR to 0.5±0.01 ms, decreased TDR (n=7, p<0.05), and suppressed TdP. Amiodarone inhibited HERG K$^+$ and late Na$^+$ currents with IC$_{50}$s of 0.8±0.1 and 3.0±0.9 μM, respectively. Conclusion: In hearts in which late I$_{Na}$ is augmented to mimic congenital or acquired pathological conditions, amiodarone has a concentration-dependent biphasic effect to induce and then suppress arrhythmic activity, secondary to inhibition of HERG K$^+$ and late Na$^+$ currents. This is the first preclinical model demonstrating the potential for amiodarone to induce TdP.

Keywords: antiarrhythmic agents; arrhythmia (mechanism); ion channels; long QT syndrome; membrane potentials.
1. INTRODUCTION

Amiodarone has long been used to treat atrial and ventricular arrhythmias\textsuperscript{1} and is reported to decrease mortality in patients with structural heart disease.\textsuperscript{1-3} The pharmacological actions of amiodarone are complex. Amiodarone has the electrophysiological characteristics of all four classes of antiarrhythmic agents, because it blocks rapidly and slowly activating delayed rectifier K\textsuperscript{+} currents (I\textsubscript{Kr} and I\textsubscript{Ks}), Na\textsuperscript{+} current (I\textsubscript{Na}), L-type Ca\textsuperscript{2+} current (I\textsubscript{CaL}) and adrenergic receptors.\textsuperscript{4}

Although acute administration of amiodarone does not increase the QTc interval, cases of TdP with acute administration of the drug have been reported.\textsuperscript{5-7} Recently, Lehtonen and colleagues\textsuperscript{5} reported 17 cases of drug-induced TdP, of which 6 cases were induced by acute (intravenous) administration of amiodarone. In another study of 23 patients with the SCN5A polymorphism S1102Y, three developed TdP while medicated with amiodarone.\textsuperscript{8} This finding is somewhat surprising because amiodarone is reported to inhibit peak and late I\textsubscript{Na} and hence is expected to reduce the arrhythmogenic effect of the S1102Y polymorphism to increase late I\textsubscript{Na}. An increased incidence of TdP among patients treated with I\textsubscript{Kr} blocking agents and who have an SCN5A polymorphism that causes a “gain of function” of late sodium channel current (late I\textsubscript{Na}) has also been reported.\textsuperscript{8-10} A reduction of repolarization reserve was proposed to explain the increased risk of TdP in patients with gain of function polymorphisms or mutations in SCN5A who are being treated with drugs that inhibit I\textsubscript{Kr}.\textsuperscript{9,11,12}

In experimental cardiac preparations, chronic use of amiodarone is associated with prolongations of the QT interval and duration of the ventricular action potential. To our knowledge, the proarrhythmic effect of either acute or chronic administration of amiodarone has not been demonstrated in experimental animal models.\textsuperscript{13-15} Regardless, the proarrhythmic
activities of I_K-blocking drugs with a recognized but low risk of prolonging the QT interval have been demonstrated in female rabbit hearts exposed to a low concentration of the sea anemone toxin II (ATX-II), which increases late I_{Na} and thus mimics the gain of function of Na^+ channels caused by some congenital SCN5A mutations and polymorphisms. An increase of late I_{Na} both reduces repolarization reserve and may lead to cellular calcium overload, which is proarrhythmic. The objective of this study was to simulate and define the mechanisms responsible for the clinical observations that acute (i.v.) administration of amiodarone can cause TdP. Although rare, this observation has yet to be explained.

2. METHODS

2.1. Female Rabbit Isolated Heart Model

This investigation conformed with the “Guide for the Care and Use of Laboratory Animals” published by the US National Institutes of Health (NIH publication No. 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 2.5-3.5 kg, were sedated then anesthetized using i.m. and i.v. injections, respectively, of xylazine and ketamine. The heart was excised and placed in a modified Krebs-Henseleit (K-H) solution (pH 7.4, gassed with 95 % O_2 and 5 %CO_2). The K-H solution contained (in mM): 118 NaCl, 2.8 KCl, 1.2 KH_2PO_4, 2.5 CaCl_2, 0.5 MgSO_4, 2.0 pyruvate, 5.5 glucose, 0.57 Na_2EDTA and 25 NaHCO_3. 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. Complete atrioventricular (AV) block was induced by thermoablation of the AV 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...
digital stimulation generator (EP MedSystems, West Berlin, NJ). After initiation of ventricular pacing, a 10-20 min period was allowed for equilibration of heart rhythm. The total duration of the experimental protocol was ≤ 3 h.

Monophasic action potential (MAP) recording

Two 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 and on the apex area of the left ventricle. One MAP electrode was placed on the endocardium of the left ventricle through a trans-septal pathway from the right ventricle. Transmural MAPD\textsubscript{90} was calculated as the difference of values of endocardial and epicardial MAPD\textsubscript{90} measured from the base of the left ventricular free wall. MAP signals were displayed in real time and digitized to determine the duration of the MAP at the level at which repolarization is 90% completed (MAPD\textsubscript{90}). Steady-state responses to drug(s) are reported in this study.

Beat-to-beat variability of MAPD\textsubscript{90} (BVR)

Values of MAPD\textsubscript{90} for 30 consecutive beats were used for calculation of the BVR of ventricular repolarization. The mean orthogonal distance on the Poincaré plot from the diagonal to each point was determined for a 30-beat interval using the following equation: \[ \frac{\sum |MAPD_{n+1} - MAPD_n|}{30 \times \sqrt{2}}.\]

12-lead ECG recording

A 12-lead electrocardiogram (ECG) from an isolated heart was recorded using a circular Einthoven-Goldberger ECG electrode system (Harvard Apparatus, Inc. Holliston, MA) connected to a Biopac Wilson ECG amplifier (Biopac, Goleta, CA). ECG parameters, such as the duration of QT interval and the duration of the T wave from Tpeak to Tend (Tpeak-Tend), were calculated using the leads with the best monophasic T wave signals. QT dispersion was
measured as the difference between the longest and the shortest QT intervals of a heart beat recorded during a steady-state response to drug in 12-lead ECG record.

**Determination of pro-arrhythmic activity of amiodarone in the absence and presence of ATX-II**

Ectopic ventricular beats (EVBs), early after-depolarizations (EADs) and ventricular tachycardia (VT), were monitored continuously during drug treatment of a heart. Post-drug control values of MAPD were obtained after drug washout. An EVB was defined as a spontaneous beat occurring earlier than the next paced beat. The maximal number of EVBs in one minute was counted as beats per minute (bpm). VT was defined as a sequence of 3 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 MAP signal.

A 3- or 5-sec pause in ventricular electrical stimulation was used to induce pause-triggered EVBs, EADs and VT in the absence and presence of drugs (ATX-II and ATX-II + amiodarone). Pauses were repeated 3 times in the presence of each concentration of test drug. Pause-triggered EADs and ventricular arrhythmias were defined as EAD, EVBs or VT that occurred within the first 3 beats after ventricular pacing was resumed.

**Determination of concentration-response relationships for effects of amiodarone on electrophysiological parameters in the absence and presence of ATX-II**

Hearts were exposed to increasing concentrations of amiodarone (1 nM-10 µM), in a cumulative manner, allowing 7-15 min between increases in drug concentration to facilitate the recording of a steady state, maximal effect. To test the effects of amiodarone in the presence of ATX-II, hearts were perfused with 3 nM ATX-II for 20 min and then exposed to amiodarone in the continued presence of 3 nM ATX-II.
2.2. Recording of electrophysiological effects of amiodarone on HEK 293 cells expressing 

\textit{SCN5A Na}^+ \text{ and } \textit{HERG (KCNH2) K}^+ \text{ channels}

Heterologous expression of \textit{SCN5A} \text{ Na}^+ \text{ and } \textit{HERG} \text{ K}^+ \text{ channels}: Human embryonic kidney (HEK293) cells stably expressing either the human \text{α-subunit of SCN5A} (purchased from Cytomyx, Cambridge, UK) or the \text{HERG K}^+ \text{ channel} (purchased from Dr. Craig T. January, University of Wisconsin-Madison, WI) were used and were maintained as previously described.\textsuperscript{19}

Voltage-clamp technique: For recording peak and late \text{I}_\text{Na}, cells were superfused with bath solution containing (in mM): 140 NaCl, 4.0 KCl, 1.8 CaCl\textsubscript{2}, 0.75 MgCl\textsubscript{2}, and 5 HEPES (pH adjusted to 7.4 with NaOH). The pipette solution contained (in mM): 20 CsCl, 120 CsF, 2 EGTA and 5 HEPES (pH adjusted to 7.4 with CsOH). For recording \text{HERG K}^+ \text{ current} (\text{I}_\text{HERG}), cells were superfused with bath solution containing (in mM): 137 NaCl, 4.0 KCl, 1.8 CaCl\textsubscript{2}, 5 HEPES and 10 glucose (pH adjusted to 7.4 with NaOH). The pipette solution contained (in mM): 130 KCl, 1.0 MgCl\textsubscript{2}, 5 EGTA, 5 MgATP and 10 HEPES (pH adjusted to 7.2 with KOH). All experiments were performed at 23±1°C.

Whole-cell membrane current was recorded as previously described.\textsuperscript{20} Briefly, patch-clamp electrodes with a resistance of 1-1.5 MΩ were made from borosilicate glass capillaries (World Precision Instruments, Sarasota, FL) using a DMZ-Universal puller (Dagan, Minneapolis, MN). Computer software (pCLAMP 9.0, Molecular Devices, Sunnyvale, CA) was used to generate voltage clamp protocols. Patch clamp amplifier (Axopatch 200B, Molecular Devices) data sampling rates varied from 5 to 100 kHz, depending on the ion channel studied. Whole-cell capacitance was compensated using the internal voltage-clamp circuitry and ~75-80% of series resistance was compensated. Membrane potentials were not corrected for junction
potentials that arise between the pipette and bath solution. To minimize possible voltage errors, small HEK293 cells of <20 pF cell capacitance (membrane capacitance; $C_m = 14.96 \pm 0.81$, n = 13), expressing peak $I_{Na}$ amplitudes of <10 nA were selected and cells were held at -140 mV and dialyzed for about 5 min before $I_{Na}$ recording. The reversal potential of $I_{Na}$ was +60 mV. Data analysis of all measured currents was performed using pCLAMP 9.0 and Origin 7.0 (MicroCal, Northampton, MA) software.

To measure the extent of tonic block (first-pulse) by amiodarone of peak $I_{Na}$, 24-ms depolarizing steps to -20 mV from a holding potential of -140 mV were applied to cells at a rate of 0.1 Hz. The magnitude of peak $I_{Na}$ in the presence of amiodarone was normalized to the respective control value.

To measure the effect of amiodarone on late $I_{Na}$, the normally small late $I_{Na}$ was augmented by exposure of cells to 3 nM ATX-II, and the effect of amiodarone to reduce the ATX-II-induced late $I_{Na}$ was determined. Late $I_{Na}$ was defined as the magnitude of $I_{Na}$ between 650 and 700 ms after application of a 700-ms depolarizing step to -20 mV from a holding potential of -140 mV applied at a rate of 0.1 Hz.

To study the concentration-response relation for inhibition of $I_{HERG}$ by amiodarone, $I_{HERG}$ was activated with a 4-sec depolarizing step to 20 mV, and tail current was recorded following a 5.7-sec repolarizing step to -50 mV. Reductions of peak tail $I_{HERG}$ by increasing concentrations of amiodarone were normalized to the respective control (no drug) values of current and plotted as relative current amplitude.

2.3. Data Analysis

Data are reported as mean ± SEM. Concentration-response relationships were analyzed using Prism version 3.0 (GraphPad, San Diego, CA). Data from experiments to measure effects
of amiodarone to inhibit peak and late $I_{Na}$ and $I_{HERG}$ were fit with the Hill equation:

$$\frac{I_{drug}}{I_{control}} = \frac{1}{1 + \left(\frac{D}{IC_{50}}\right)^n},$$

where $I_{drug}/I_{control}$ is fractional block, $D$ is drug concentration, $IC_{50}$ is half-maximal inhibitory concentration and $n$ is the Hill coefficient ($n_H$). To compare values of measurements obtained from the same heart before and after a drug treatment, repeated measures one-way analysis of variance (ANOVA) was used and Student-Newman-Keuls test was applied to determine which pairs of group means were significantly different. Paired and non-paired Student t-tests were used to determine the significance of a difference between two means before (as control) and after drug treatment in the same or different hearts, respectively. A significant difference between 2 group means was defined as one having a $p<0.05$.

2.4. Materials

Amiodarone (Sigma-Aldrich, MO, USA) was dissolved in dimethylsulfoxide (DMSO) at concentration of $2 \times 10^{-2}$ M as stock in $-4 \, ^\circ\text{C}$, and further diluted to $4 \times 10^{-4}$ M in physiological saline for use in experiments. The final content of DMSO in saline during experiments was not more than 0.05%. ATX-II (Alomone Labs, Israel) was dissolved in normal saline.

3. RESULTS

3.1. Proarrhythmic effects of amiodarone in the presence of ATX-II in female rabbit isolated hearts

Amiodarone alone (0.01-10 µM) did not caused EVBs, EAD or VT (Figs. 1 and 3, Table). At a concentration of 30 nM, amiodarone caused small and significant ($p<0.05$) increases in BVR, $T_{peak-Tend}$ and the index of $T_{peak-Tend}/QT$ interval, but did not significantly prolong either epicardial or endocardial MAPD$_{90}$, or transmural dispersion of MAPD$_{90}$ ($n=6-9$, $p>0.05$, Fig. 3 and Table). At a concentration of 10 µM, amiodarone caused a small and significant ($p<0.05$) shortening of epicardial and endocardial MAPD$_{90}$ and a reduction of dispersion of
MAPD$_{90}$ (Fig. 3). Amiodarone (10 µM) prolonged the QT (Fig. 3) and QRS intervals but did not change the JT interval and QT dispersion (Table).

ATX-II (3 nM) alone caused infrequent EVBs (Fig. 2B) and short episodes of TdP in 2 of 17 (12%) hearts (Fig. 1) and significantly prolonged the epicardial and endocardial MAPD$_{90}$ (n=16, p<0.001, Fig. 3 and Table), transmural dispersion (endo-epi) of MAPD$_{90}$, BVR, Tpeak-Tend, QT interval and QT dispersion and JT interval, as well as the index of Tpeak-Tend/QT interval (Fig. 3, Table). ATX-II (3 nM) caused no change in the QRS interval (p>0.05; Table). ATX-II was subsequently used to sensitize the rabbit heart to amiodarone, in an attempt to unmask the proarrhythmic potential of the drug.$^{16}$

In the presence of 3 nM ATX-II, a stepwise increase in the concentration of amiodarone was associated with a biphasic response, initially inducing arrhythmic activity and then suppressing it (Figs. 1, 2 and 3). Episodes of spontaneous TdP were observed in 16 of 17 (94%) hearts exposed to amiodarone at concentrations of 30-300 nM (Figs. 1 and 2C) but were not observed in any of these same hearts when the concentration of amiodarone was increased to 10 µM (Figs. 1 and 2D). EVBs and VTs triggered by 3- or 5-sec pauses were seen in 6 out of 8 (75%) hearts when the concentration of amiodarone was increased from 1 to 30-60 nM (Fig. 2G). Neither spontaneous nor pause-triggered TdP was observed when the amiodarone concentration was 3-10 µM (Figs. 1, 2D, 2H). The maximum number of EVBs per minute was significantly increased by 30 nM amiodarone in the presence of 3 nM ATX-II from 3 ± 2 to 13 ± 3 bpm (n=8, p<0.001), but decreased to 4 ± 1 bpm when the concentration of amiodarone was further increased to 10 µM (Table).

In the continuous presence of 3 nM ATX-II, amiodarone at a concentration of 30 nM caused significant (n=6-16, p<0.05-0.001 compared to ATX-II alone) increases in epicardial and
endocardial MAPD$_{90}$ (Fig. 3A), transmural dispersion of MAPD$_{90}$, QT interval prolongation (Fig. 3B), BVR (Fig. 3C), Tpeak-Tend (Fig. 3D), index of Tpeak-Tend/QT interval, QT dispersion and JT intervals (Table). However, at concentrations of 10 µM, amiodarone shortened epicardial MAPD$_{90}$ (n=6, Fig. 3A and Table 1, p<0.01), and decreased transmural MAPD$_{90}$ dispersion (Table 1), QT interval prolongation (Fig. 3B and Table), BVR (Fig. 3C and Table), Tpeak-Tend (Fig. 3D and Table), index of Tpeak-Tend/QT interval and JT intervals, (n=5-16, p<0.05-0.001, Table). Polymorphic ventricular tachycardias (TdP) occurred at the peak of the concentration-response relationship for amiodarone in the presence of 3 nM ATX-II (i.e., at 10-300 nM amiodarone; Figs. 1, 2 and 3).

3.2 Reproducibility and reversibility of ventricular arrhythmias in the presence of ATX-II

The proarrhythmic effect of amiodarone in the presence of ATX-II was reversible and reproducible. As shown in Fig. 2J, in a group of 6 hearts, 3 nM ATX-II caused occasional EVBs but no VT. In the presence of 3 nM ATX-II, amiodarone (60 nM) caused frequent EVBs (18 ± 4 bpm, n=6), EADs and VTs in all 6 hearts (Fig. 2K). Following washout of amiodarone (in the continued presence of ATX-II), there was a decrease in the number of EVBs 3 ± 2 bpm (n=6, p<0.01) and no VT was observed (Fig. 2L, Table). In the continued presence of 3 nM ATX-II, reintroduction of amiodarone again led to an increase of EVBs to 28 ± 6 bpm and polymorphic VT in all 6 of 6 hearts tested (Fig. 2M and Table).

3.3 Effects of amiodarone on peak and late I$_{Na}$ and I$_{HERG}$ in HEK 293 cells

Amiodarone inhibited both late I$_{Na}$ that was induced by ATX-II and peak I$_{Na}$ [peak I$_{Na}$ was recorded in the absence of ATX-II] (Fig. 4, A and B). A representative record of the effect of amiodarone to reduce late I$_{Na}$ in the presence of 3 nM ATX-II is shown in Fig. 4A. The IC$_{50}$ and n$_{H}$ values for inhibition of late I$_{Na}$ by amiodarone were 3.0 ± 0.9 µM and 0.6 ± 0.2,
respectively (Fig. 4B). The IC$_{50}$ and n$_H$ values for tonic block of peak $I_{Na}$ by amiodarone were 178.1 ± 17.2 $\mu$M and 1.5 ± 0.2, respectively. The magnitude of peak tail $I_{HERG}$ was also reduced by amiodarone (Fig. 4, C and D). The IC$_{50}$ and n$_H$ values for reduction of peak tail $I_{HERG}$ by amiodarone were 0.8 ± 0.1 $\mu$M and 1.3 ± 0.2, respectively.

3.4 Correlation of $I_{HERG}$ inhibition and proarrhythmic risk in the presence of ATX-II

In HEK293 cells, 0.1 $\mu$M amiodarone inhibited $I_{HERG}$ by 13 ± 4 % (Fig. 4D, inset). In the presence of 3 nM ATX-II, 0.1 $\mu$M amiodarone caused TdP in rabbit isolated hearts (Figs. 1, 2 and 3). To confirm that a small (~13%) inhibition of $I_{HERG}$ by amiodarone in the presence of 3 nM ATX-II may be sufficient to cause TdP, the effect of a low concentration of E-4031, a pure $I_{Kr}$ blocking agent, on rabbit isolated hearts in the absence and presence of 3 nM ATX-II was determined. E-4031 at a concentration of 1 nM caused a 10% inhibition of $I_{HERG}$ similar to 0.1 $\mu$M amiodarone (Fig. 4D, inset). In female rabbit isolated heart, E-4031 (1 nM) alone caused neither MAPD prolongation (n=9, p>0.05) nor TdP. However, in presence of 3 nM ATX-II, E-4031 (1 nM) caused a significant increase in MAPD$_{90}$ from 300 ± 18 ms to 361 ± 10 ms (n=8, p<0.01) and TdP in 5 of 8 hearts (not shown).

4. DISCUSSION

Amiodarone is known to have both anti- and pro-arrhythmic effects in patients and its use is associated with a low incidence of TdP. It has been difficult to study these effects because they are not easily mimicked in animal preparations. The results of this study indicate that acute proarrhythmic activities of amiodarone can be reliably unmasked when late $I_{Na}$ is increased by ATX-II. Low concentrations of amiodarone (e.g., 30 nM) that alone caused no significant APD prolongation or TdP did cause significant APD prolongation and TdP when administered in combination with 3 nM ATX-II. Furthermore, although 30 nM amiodarone alone did not cause
significant prolongation of APD, it did significantly increase BVR, Tpeak-Tend and the index of Tpeak-Tend/QT interval, suggesting that these three parameters are more sensitive than QT interval and AP duration to detect the proarrhythmic potential of amiodarone. The results are consistent with the recent report of amiodarone-induced TdP in patients with the SCN5A polymorphism S1102Y. An increase of late I_{Na} is associated with a wide variety of pathophysiological conditions. The risk of ventricular arrhythmic activity in patients with these conditions or with gain-of-function SCN5A polymorphisms or mutations may be expected to increase during administration of low concentrations of amiodarone (this study) and other I_{HERG} blocking agents.

The occurrence of TdP in rabbit hearts exposed to amiodarone in the presence of 3 nM ATX-II can be attributed to amiodarone because: 1) TdP induced by a low concentration of amiodarone could be suppressed by increasing the concentration of amiodarone in the presence of a fixed concentration of ATX-II; and 2) the incidence of TdP in the presence of 3 nM ATX-II alone was much lower (2 of 17 hearts) than in the presence of both ATX-II and amiodarone (16 of 17 hearts). However, the risk factors for TdP are multiple. The observation that the combination of low concentrations of ATX-II and amiodarone yielded a high incidence of TdP in rabbit heart is consistent with the clinical observation that TdP occurs when multiple risk factors (congenital and acquired) are present.

Reported acute effects of amiodarone on APD in single cell preparations from different tissues and animal species vary. In the female rabbit isolated heart, amiodarone alone induced only small changes in electrophysiological parameters (see Table) and did not cause TdP. However, in the presence of ATX-II, which increases late I_{Na} and thereby reduces repolarization reserve, both anti- and pro-arrhythmic, concentration-dependent effects of amiodarone were
observed. The proarrhythmic effect of amiodarone occurred at lower concentrations (30-300 nM) than the anti-arrhythmic effect, and at concentrations lower than the therapeutic range of 0.5-7.8 µM. Amiodarone (30 nM) in the presence of ATX-II not only caused frequent EVBs, EADs and polymorphic VTs, but also increased the transmural dispersion in MAPD\(_{90}\) (endocardial MAPD\(_{90}\)-epicardial MAPD\(_{90}\)), BVR of MAPD\(_{90}\), Tpeak-Tend and the index of Tpeak-Tend/QT interval. These electrophysiological changes correlated with the occurrence of ventricular arrhythmias, which is consistent with the knowledge that BVR, transmural dispersion of ventricular repolarization and the index of Tpeak-Tend/QT interval are important determinants or markers of the proarrhythmic effects of QT-prolonging drugs. When the concentration of amiodarone was increased to 1-10 µM in the presence of ATX-II, the ventricular arrhythmias, and increases in MAPD, transmural MAP dispersion, QT interval (JT interval), Tpeak-Tend, index of Tpeak-Tend/QT interval and BVR were reduced.

The biphasic (pro- and anti- arrhythmic) concentration-dependent effects of amiodarone are best explained by inhibition of a different combination of ion channels at low versus high concentrations of the drug. At a low concentration (about 0.1 µM), amiodarone is a relatively pure \(I_{Kr}\) blocking agent. The synergistic effects of selective \(I_{Kr}\) inhibition at these low concentrations of amiodarone (or E-4031) and the ATX-induced increase in late \(I_{Na}\) would be expected to reduce repolarization reserve, resulting in a proarrhythmic effect. In fact, as shown here, in the presence of 3 nM ATX-II relatively small reductions of \(I_{Kr}\) by amiodarone and E-4031 (13 and 10%, respectively), were sufficient enough to cause TdP. Thus, patients with congenital (e.g., LQT3) or acquired pathological conditions (e.g., structural heart disease) associated with an increase in late \(I_{Na}\) may be at increased risk of proarrhythmia when \(I_{Kr}\) is inhibited by as little as 10%. Furthermore, amiodarone has been shown to preferentially bind to
the inactivated state of the cardiac sodium channel.\textsuperscript{26} The inhibition by amiodarone of late $I_{\text{Na}}$ serves to counterbalance the effect of inhibition of $I_{\text{Kr}}$, thus reducing the drug-induced decrease in net repolarizing current (repolarization reserve). The IC\textsubscript{50} values for amiodarone inhibition of $I_{\text{HERG}}$ and late $I_{\text{Na}}$ in HEK 293 cells expressing either $HERG$ or $SCN5A$ were $0.8 \pm 0.1$ and $3.0 \pm 0.9$ μM, respectively. IC\textsubscript{50} values for amiodarone-induced inhibition of $I_{\text{Kr}}$ and late $I_{\text{Na}}$ in rabbit and human hearts were reported to be $2.8$ and $6.7$ μM, respectively.\textsuperscript{26, 27} The IC\textsubscript{50} values reported in our study have to be interpreted with caution as the Hill coefficient fit was forced to zero. Nevertheless, at therapeutic concentrations (1-10 μM), amiodarone inhibits both $I_{\text{Kr}}$ and late $I_{\text{Na}}$, and this may be responsible, at least in part, for the antiarrhythmic effects and the low risk of long-QT-related arrhythmias attending common use of the drug. High concentrations of amiodarone also inhibit peak $I_{\text{Na}}$ in a use-dependent manner\textsuperscript{28}, and this may explain amiodarone’s effect to increase the QRS interval in the female rabbit heart (see table). Similarly, cisapride was also shown to have the biphasic concentration/response relationship to induce long-QT syndrome and TdP.\textsuperscript{29}

Our findings support reports that an abnormal increase of late $I_{\text{Na}}$ due either to heritable $SCN5A$ mutations,\textsuperscript{8, 22} structural heart disease (e.g., heart failure\textsuperscript{30-32}), or exposure to reactive oxygen species may diminish the repolarization reserve of the myocardium and lead to an increased susceptibility to drug-induced TdP. For example, amiodarone- and sotalol-induced repeated episodes of TdP were reported in patients with $KCNQ1$ and $KCNH2$ mutations,\textsuperscript{5} and amiodarone-induced TdP appears to be more common in subjects with inherited mutations or structural heart diseases, including congestive heart failure and dilated cardiomyopathy.\textsuperscript{33, 34}

5. Study Limitations:
1) This study is concerned with the acute effects of amiodarone and these are likely to differ from those seen during chronic therapy with the drug; 2) the calcium channel blocking effects of amiodarone may contribute in part to the shortening of MAPD at high concentrations (4-10 μM) but were not investigated; 3) the pacing rate of 1 Hz, although chosen to increase the sensitivity of the rabbit heart to the proarrhythmic effect of amiodarone (i.e., bradyarrhythmia is a risk factor for TdP), is a slow rate and the proarrhythmic effects of amiodarone at normal or higher pacing rates are expected to be less than at 1 Hz.

6. CONCLUSION

A proarrhythmic effect of amiodarone was observed at low concentrations (30-300 nM) of the drug at which the APD was prolonged in the presence but not in the absence of ATX-II. BVR, Tpeak-Tend and the index of Tpeak-Tend/QT interval were better predictors of the proarrhythmic potential of amiodarone than was the magnitude of APD prolongation. An anti-arrhythmic effect of amiodarone was observed in the presence of ATX-II during administration of higher concentrations (1-10 μM) of the drug. The biphasic pro- and anti-arrhythmic effects of amiodarone appear to reflect the action of the drug to inhibit I_{Kr} at lower concentrations than it inhibits I_{Na}. A possible implication of our finding is that a drug that causes minimal (e.g., ≤10 ms) prolongation of QTc interval in an otherwise normal heart may be proarrhythmic in hearts with acquired or congenital reductions in repolarization reserve. Consequently the absence of a drug action to prolong the QTc interval in a heart with normal repolarization reserve is not a reliable predictor of drug safety.

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REFERENCES


Table. Effect of amiodarone on EP parameters in absence and presence of ATX-II

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>30 nM</th>
<th>10 µM</th>
<th>Alone</th>
<th>30 nM</th>
<th>10 µM</th>
</tr>
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<tbody>
<tr>
<td>Epi- MAPD&lt;sub&gt;90&lt;/sub&gt;</td>
<td>185 ± 5  (n=9)</td>
<td>190 ± 4 (n=7)</td>
<td>175 ± 9 (n=9)*</td>
<td>217 ± 8 (n=16)**</td>
<td>237 ± 6 (n=16)††</td>
<td>185 ± 8 (n=15)‡‡</td>
</tr>
<tr>
<td>Endo- MAPD&lt;sub&gt;90&lt;/sub&gt;</td>
<td>208 ± 7  (n=8)</td>
<td>214 ± 4 (n=7)</td>
<td>183 ± 12 (n=9)**</td>
<td>276 ± 12 (n=16)**</td>
<td>313 ± 14 (n=16)††</td>
<td>221 ± 11 (n=15)‡‡</td>
</tr>
<tr>
<td>TDR</td>
<td>22 ± 4   (n=8)</td>
<td>36 ± 4 (n=7)</td>
<td>5 ± 13 (n=8)</td>
<td>59 ± 9 (n=16)**</td>
<td>77 ± 10 (n=16)††</td>
<td>40 ± 8 (n=15)‡‡</td>
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<tr>
<td>BVR</td>
<td>0.34 ± 0.01 (n=9)</td>
<td>0.45 ± 0.04 (n=7)*</td>
<td>0.39 ± 0.02 (n=9)</td>
<td>0.75 ± 0.03 (n=6)**</td>
<td>1.06 ± 0.13 (n=6)†</td>
<td>0.50 ± 0.01 (n=6)‡‡</td>
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<tr>
<td>Tpeak-Tend</td>
<td>33 ± 2   (n=8)</td>
<td>40 ± 3 (n=8)*</td>
<td>39 ± 3 (n=6)</td>
<td>64 ± 7 (n=9)**</td>
<td>95 ± 9 (n=9)††</td>
<td>54 ± 7 (n=9)‡‡</td>
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<tr>
<td>QT Interval</td>
<td>249 ± 7  (n=8)</td>
<td>255 ± 9 (n=8)</td>
<td>257 ± 10 (n=6)*</td>
<td>335 ± 18 (n=10)**</td>
<td>385 ± 27 (n=10)†</td>
<td>295 ± 16 (n=10)‡‡</td>
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<tr>
<td>Tpeak-Tend/QT Interval</td>
<td>0.13 ± 0.01 (n=8)</td>
<td>0.16 ± 0.01 (n=8)**</td>
<td>0.14 ± 0.01 (n=8)</td>
<td>0.19± 0.01 (n=9)**</td>
<td>0.24 ± 0.01 (n=9)††</td>
<td>0.18 ± 0.02 (n=9)‡‡</td>
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<tr>
<td>QT Dispersion</td>
<td>22 ± 1   (n=13)</td>
<td>22 ± 1 (n=6)</td>
<td>22 ± 1 (n=6)</td>
<td>38 ± 1 (n=7)**</td>
<td>47 ± 4 (n=7)††</td>
<td>27 ± 2 (n=7)‡‡</td>
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<tr>
<td>JT Interval</td>
<td>176 ± 7  (n=8)</td>
<td>176 ± 7 (n=8)</td>
<td>178 ± 7 (n=7)</td>
<td>279 ± 19 (n=9)**</td>
<td>328 ± 28 (n=9)††</td>
<td>225 ± 17 (n=9)‡‡</td>
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<tr>
<td>V ERP</td>
<td>150 ± 8  (n=6)</td>
<td>153 ± 13 (n=6)</td>
<td>180 ± 7 (n=6)*</td>
<td>204 ± 14 (n=8)*</td>
<td>226 ± 15 (n=8)*</td>
<td>219 ± 9 (n=8)*</td>
</tr>
<tr>
<td>VAT</td>
<td>33 ± 1   (n=7)</td>
<td>34 ± 2 (n=6)</td>
<td>35 ± 2 (n=6)*</td>
<td>30 ± 1 (n=9)</td>
<td>30 ± 1 (n=9)</td>
<td>33 ± 1 (n=9)</td>
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<tr>
<td>QRS Interval</td>
<td>74 ± 2   (n=8)</td>
<td>76 ± 2 (n=8)</td>
<td>80 ± 2 (n=7)*</td>
<td>74 ± 3 (n=10)</td>
<td>78 ± 3 (n=10)</td>
<td>82 ± 4 (n=10)‡‡</td>
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<tr>
<td>EVBs</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5 ± 3</td>
<td>19 ± 4 †</td>
<td>3 ± 2‡</td>
</tr>
</tbody>
</table>

Summary of electrophysiological parameters in hearts exposed to either amiodarone alone (30 nM and 10 µM) or ATX-II (3 nM) plus amiodarone. *, **, †, ††, and ‡, ‡‡ indicate p<0.05 or p<0.01 compared to control, 3 nM ATX-II, and 3 nM ATX-II + 30 nM amiodarone, respectively. TDR: transmural dispersion (endocardial-epicardial) of MAPD<sub>90</sub>; BVR: beat-to-beat variability of MAPD<sub>90</sub>; VERP: ventricular effective refractory period; VAT: ventricular activation time; EVB: ectopic ventricular beat.
FIGURE LEGENDS

Fig. 1. Concentration-dependent pro- and anti- arrhythmic effects of amiodarone in the absence and presence of 3 nM ATX-II in a female rabbit isolated heart paced at 1 Hz. Bars indicate the incidence of ventricular tachycardia (torsade de pointes, TdP) in presence of amiodarone alone (left panel) and ATX-II (3 nM) plus amiodarone (right panel). Numbers in parentheses are hearts with VT/total number of hearts studied.

Fig. 2. Representative recordings of concentration-dependent, biphasic pro- and anti- arrhythmic effects of amiodarone in presence of 3 nM ATX-II in a female rabbit isolated heart paced at 1 Hz. In each panel monophasic action potentials (top record in each panel) and electrocardiogram (bottom record) were simultaneously recorded. From A to D: Hearts were serially exposed to (A) control (no drug), (B) ATX-II (3 nM) alone, (C) ATX-II (3 nM) + amiodarone (60 nM) and (D) ATX-II (3 nM) + amiodarone (10 µM), respectively. From E to H: Pause-triggered amiodarone-induced arrhythmias in presence of 3 nM ATX-II were observed in 6 out of 8 hearts. From I to M: Reversibility and reproducibility of proarrrhythmic effect of amiodarone in the continued presence of ATX-II (3 nM). The heart was serially exposed to (I) control (no drug), (J) ATX-II (3 nM) alone, (K) ATX-II (3 nM) + amiodarone (60 nM), (L) ATX-II (3 nM) after termination of amiodarone infusion, and (M) ATX-II (3 nM) + amiodarone (60 nM) after re-infusion of amiodarone (60 nM), respectively. The duration of MAPs was shortened after the occurrence of either frequent ectopic ventricular beats or short ventricular tachycardias (panels C, J, K and M).

Fig. 3. Concentration-response relationships for amiodarone on MAPD90 (A), QT interval (B), beat-to-beat variability (C) and ECG Tpeak-Tend (D) in female rabbit isolated hearts in the absence and presence of 3 nM ATX-II (n=6 in each group). Values were calculated as the changes from the control in individual hearts and are represented as mean ± SEM. Asterisks indicate the significance (p<0.05) of the difference from either control (amiodarone alone) or ATX-II (ATX-II + amiodarone). † indicates the occurrence of polymorphic ventricular tachycardia (TdP). Baseline values for these parameters are listed in the table.

Fig. 4. Inhibition of peak and late I_{Na}, and peak tail I_{HERG} by amiodarone. Panel A: The voltage clamp protocol (top) and a representative recordings of late I_{Na} from a single cell in the absence
of drug (control, a), during superfusion with 3 nM ATX-II (b), and during superfusion with 0.3 (c) and 3 µM (d) amiodarone in the continued presence of 3 nM ATX-II. Inset shows representative recordings of peak $I_{Na}$ from a single cell in the absence (control), and in the presence of increasing concentrations of amiodarone. Scale bars represent 1 msec and 1 nA, respectively. Panel B: Concentration-response relations for inhibition of peak and late $I_{Na}$ by amiodarone. Panel C: $I_{HERG}$ traces from a single cell exposed to 0 (control), 0.3, 1 and 3 µM amiodarone. Panel D: Concentration-response relation for inhibition of peak tail $I_{HERG}$ by amiodarone. Inset shows $I_{HERG}$ inhibitions by 100 nM amiodarone and 1 nM E-4031. Number of determinations is indicated in parentheses.
Fig. 1

Incidence of TdP (%)

Amiodarone (µM)
alone

Amiodarone (µM) in
presence of 3 nM ATX-II

(0/9)  (0/9)  (0/9)  (0/9)
0 0.01-0.01  0.03-0.3  1-10

(2/17)  (3/17)  (16/17)
0 0.001-0.01  0.02-0.3  1-10

(0/17)
A. Control

B. ATX-II 3 nM

C. ATX-II (3 nM)+amiodarone (60 nM)

D. ATX-II (3 nM)+amiodarone (10 μM)
E. Control

F. ATX-II (3 nM)

G. ATX-II (3 nM)+amiodarone (60 nM)

H. ATX-II (3 nM)+Amiodarone (10 μM)

1 sec
I. Control

J. ATX-II (3 nM) alone

K. ATX-II (3 nM)+amiodarone (60 nM)

H. ATX-II (3 nM) (washout amiodarone)

I. ATX-II (3 nM)+amiodarone (60 nM)
Fig. 3

A. MAPD$_{90}$

B. QT Interval

C. BVR

D. Tpeak - Tend

By guest on April 29, 2016 Downloaded from
Fig. 4

A. 

Late $I_{Na}$ (pA)

-140 mV

-1000

-600

-200

0

200

600

1000

700 msec

30 μM

100 μM

Control

b
c
d

B. 

Relative $I_{Na}$

0.0

0.5

1.0

Late $I_{Na}$

Peak $I_{Na}$

Amiodarone conc (μM)

(3)

(4)

(5)

(4)

(4)

(5)

(4)

C.

$I_{Na}$ (pA)

Control

0.3 μM

1 μM

3 μM

250 pA

2 sec

D. 

Relative $I_{HERG}$

0.0

0.5

1.0

$I_{HERG}$ Inhibition (%)

0%

5%

10%

15%

20%

Amiodarone (100 nM) E-4031 (1 nM)

(3)

(5)

(4)

(4)

(4)