Phase 2 Early Afterdepolarization as a Trigger of Polymorphic Ventricular Tachycardia in Acquired Long-QT Syndrome

Direct Evidence From Intracellular Recordings in the Intact Left Ventricular Wall

Gan-Xin Yan, MD, PhD; Ying Wu, MD; Tengxian Liu, BS; Jixin Wang, MS; Roger A. Marinchak, MD; Peter R. Kowey, MD

Background—This study examined the role of phase 2 early afterdepolarization (EAD) in producing a trigger to initiate torsade de pointes (TdP) with QT prolongation induced by dl-sotalol and azimilide. The contribution of transmural dispersion of repolarization (TDR) to transmural propagation of EAD and the maintenance of TdP was also evaluated.

Methods and Results—Transmembrane action potentials from epicardium, midmyocardium, and endocardium were recorded simultaneously, together with a transmural ECG, in arterially perfused canine and rabbit left ventricular preparations. dl-Sotalol preferentially prolonged action potential duration (APD) in M cells dose-dependently (1 to 100 μmol/L), leading to QT prolongation and an increase in TDR. Azimilide, however, significantly prolonged APD and QT interval at concentrations from 0.1 to 10 μmol/L but shortened them at 30 μmol/L. Unlike dl-sotalol, azimilide (>3 μmol/L) increased epicardial APD markedly, causing a diminished TDR. Although both dl-sotalol and azimilide rarely induced EADs in canine left ventricles, they produced frequent EADs in rabbits, in which more pronounced QT prolongation was seen. An increase in TDR by dl-sotalol facilitated transmural propagation of EADs that initiated multiple episodes of spontaneous TdP in 3 of 6 rabbit left ventricles. Of note, although azimilide (3 to 10 μmol/L) increased APD more than dl-sotalol, its EADs often failed to propagate transmurally, probably because of a diminished TDR.

Conclusions—This study provides the first direct evidence from intracellular action potential recordings that phase 2 EAD can be generated from intact ventricular wall and produce a trigger to initiate the onset of TdP under QT prolongation.

Key Words: depolarization • action potentials • tachycardia

Acquired long-QT syndrome induced by medical products is associated with an atypical type of polymorphic ventricular tachycardia (VT), torsade de pointes (TdP), leading to sudden cardiac death. The mechanisms responsible for the initiation and maintenance of polymorphic VT under QT prolongation, however, are not fully understood.

It has been hypothesized that phase 2 early afterdepolarization (EAD) could induce a triggered response for the initiation and a functional reentrant substrate for maintenance in the development of TdP. EAD has frequently been observed in isolated ventricular tissue or single myocytes in the presence of action potential duration (APD)–prolonging agents or lower extracellular potassium concentration. But the data obtained from in vivo animal experiments or patients are not that convincing. This is probably because a larger electrical load of myocytes in intact ventricular wall, in which all ventricular myocytes are electrically coupled, blunts the generation of any potential fluctuations, such as EAD. Technical limitations to access intramural sites and endocardium by use of intracellular action potential recording under in vivo conditions also contribute. Apparent phase 3 (instead of phase 2) EAD has been observed by use of a traditional Franz monophasic action potential (MAP) recording electrode in patients with long-QT syndrome. Such a composite electrical signal recorded with MAP electrodes from ventricular tissue by applying pressure, however, is influenced by many factors. EAD-like electrical activity recorded by this technique may represent an artifact that is secondary to local heterogeneous repolarization under conditions of QT prolongation. Therefore, the questions of whether EAD could be generated from intact ventricular wall and how it could initiate the onset of TdP still remain to be answered.
The present study uses isolated arterially perfused rabbit ventricular preparations to provide the first direct evidence from intracellular recordings that EAD could originate from any of 3 myocardial layers within intact ventricular wall under conditions of marked QT prolongation. Transmural dispersion of repolarization (TDR), which is enhanced by the blockade of $I_{Ks}$ alone by dl-sotalol and decreased by additional inhibition of $I_{Ks}$ by azimilide, plays an important role in transmural propagation of EAD (malignant “R-on-T” extrasystole). The R-on-T extrasystole can in turn initiate the development of TdP when TDR is exaggerated.

**Methods**

**Arterially Perfused Canine and Rabbit Left Ventricular Wedge Preparations**

The methods used for isolation, perfusion, and recording of transmembrane activity from the arterially perfused canine ventricular wedge preparation, as well as the viability and electrical stability of the preparation, are detailed in previous studies. The preparation of the rabbit left ventricle is principally the same as the canine left ventricular wedge. Briefly, the entire rabbit left ventricle was cannulated and perfused with Tyrode’s solution via the left main coronary artery. Both atria, the right ventricle, and the septum were removed so that the endocardium of the left ventricle was accessible to floating intracellular microelectrodes.

**Electrophysiological Recordings**

Transmembrane action potentials in wedge preparations were recorded simultaneously from epicardial, subendocardial, and endocardial sites by use of 3 separate intracellular floating microelectrodes. In some experiments with rabbit left ventricle, action potentials were recorded from only the epicardium and endocardium because of difficulty in access to the subendocardium. Our data have demonstrated, however, that there is no significant difference in APD between subendocardial and endocardial sites. This may indicate a strong electrotonic interaction between 2 cell types within the rabbit ventricular wall. A transmural ECG was recorded concurrently in all experiments.

APD was measured at 90% repolarization (APD$_{90}$). TDR was defined as the difference between the longest and shortest repolarization times across the left ventricular wall. On the ECG, TDR is approximately equal to the interval from the end to the peak of the T wave (QT$_{end}$-$QT_{peak}$). The QT interval was defined as the time from the onset of the QRS to the point at which the final downslope of the T wave crosses the isoelectric line.

**Statistics**

Statistical analyses of the data were performed with Student’s $t$ test for paired data or 1-way ANOVA coupled with Scheffé’s test as appropriate. The $\chi^2$ test was used for comparisons between 2 groups for event incidences. Data are presented as mean±SEM unless otherwise indicated. Significance was defined as a value of $P<0.05$.

**Results**

**Effects of dl-Sotalol and Azimilide on QT Interval, APD, and TDR in the Canine Left Ventricular Wedge**

The canine left ventricular wedge was used to assess dose-dependent effects of dl-sotalol and azimilide on QT interval, APD, and TDR. dl-Sotalol produced a dose-dependent prolongation of QT interval and APD at concentrations of 1 to 100 $\mu$mol/L (Figure 1A). Similar results in 4 preparations are summarized in Figure 2A. In the presence of 100 $\mu$mol/L dl-sotalol, APD increased maximally from 226.2±5.2 ms to 260.8±5.2 ms in epicardium and 263.8±1.7 to 323.0±4.8 ms in M cells at a basic cycle length (BCL) of 2000 ms. The QT prolongation of M-cell APD in presence of higher doses (0.3 to 3 $\mu$mol/L) of azimilide caused a decrease in TDR.
interval increased, in parallel with M-cell APD, by 22%, from 276.5±1.6 to 335.9±6.2 ms (P<0.01).

Preferential prolongation of M-cell APD by dl-sotalol was associated with a marked increase in TDR (Figure 3A). On the transmural ECG, such a dose-dependent increase in TDR always manifested as a positive broad and tall T wave on the ECG, reflected by an increase in the QT\textsubscript{end}-QT\textsubscript{peak} interval (Figure 1A).

Different from dose-dependent APD and QT prolongation induced by the pure I\textsubscript{Kr} blocker dl-sotalol, a dual effect of azimilide on QT interval and APD was observed (Figures 1B and 2B). Azimilide prolonged QT interval and APD at concentrations of 0.3 to 10 \textmu mol/L but shortened them at 30 \textmu mol/L, an effect probably secondary to its inhibition of L-type calcium currents at higher concentrations.\textsuperscript{6} In the presence of 10 \textmu mol/L azimilide, APD\textsubscript{90} increased maximally from 227.5±9.8 to 356.8±29.9 ms in epicardium and 264.2±6.7 to 370.4±13.6 ms in M cells (P<0.01, n=5, BCL=2000 ms). Interestingly, the QT interval at higher doses of azimilide (>3 \textmu mol/L) no longer followed M-cell APD, an effect due to preferential prolongation of epicardial APD (Figure 2B). Although azimilide 10 \textmu mol/L prolonged QT interval by 53% and M-cell APD by 40%, more significantly than the prolongations (22% and 22%, respectively) induced by dl-sotalol 100 \textmu mol/L, it did not significantly increase TDR (Figure 3A). Actually, azimilide tended to decrease TDR at concentrations >3 \textmu mol/L (Figure 3) because of preferential prolongation of epicardial APD. As expected, a decrease in TDR despite marked QT prolongation was associated with a flattened T wave, reflected by a decrease in QT\textsubscript{end}-QT\textsubscript{peak} interval (Figure 1B).

Although spontaneous TdP was observed in the canine ventricular wedge in the presence of APD-prolonging agents,\textsuperscript{3,10,11} a previous attempt to record EADs from any of the cell types (Purkinje fiber, M cells, endocardium, and epicardium) in this preparation during QT prolongation failed. Interestingly, azimilide 10 \textmu mol/L prolonged APD more significantly in epicardium than in M and endocardial cells, resulting in the generation of EADs from epicardium (BCL=2000 ms). Such preferential prolongation of APD in epicardium, however, reduced TDR so that the EADs failed to conduct transmurally (Figure 1B).

### Roles of dl-Sotalol and Azimilide in the Generation of EAD, R-on-T Extrasystole, and Spontaneous TdP in Rabbit Left Ventricle

Although EADs were rarely observed in canine left ventricular wedge under QT prolongation, this was not the case in arterially perfused rabbit left ventricle. Both dl-sotalol and azimilide produced marked QT and APD prolongation in isolated arterially perfused rabbit left ventricle, resulting in frequent EADs at a BCL=2000 ms.

The striking electrophysiological difference between dog and rabbit was that prolongation of QT interval and APD in response to dl-sotalol and azimilide was more pronounced in rabbit left ventricles, although similar patterns were observed for both agents in the canine left ventricular wedge. The effects of 100 \textmu mol/L dl-sotalol and 10 \textmu mol/L azimilide on APD\textsubscript{90} and QT intervals in rabbit left ventricle are shown in Table 1.

As shown in Figure 4, dl-sotalol 50 \textmu mol/L produced EADs in subendocardium and endocardium, inducing an R-on-T extrasystole on the ECG. In the present study, transmembrane action potentials from epicardial and endocardial sites were recorded simultaneously, and dynamic changes in the shape and size of EADs could be monitored continuously. Therefore, the origin of EADs can be approximately located on the basis of the appearance of the earliest EAD in each recording. Mapping the exact origin of EAD

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**Table 1. Effects of dl-Sotalol and Azimilide on QT Interval, APD\textsubscript{90}, Phase 2 EAD, R-on-T Extrasystole, and TdP in Isolated Arterially Perfused Rabbit Left Ventricle**

<table>
<thead>
<tr>
<th></th>
<th>APD\textsubscript{90}, ms</th>
<th>Epicardium</th>
<th>Subendocardium</th>
<th>Endocardium</th>
<th>QT, ms</th>
<th>EAD, n</th>
<th>R-on-T Extrasystole, n</th>
<th>TdP, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>209.0±13.6</td>
<td>243.8±16.5</td>
<td>244.3±18.3</td>
<td>258.0±18.4</td>
<td>0 /6</td>
<td>0 /6</td>
<td>0 /6</td>
<td></td>
</tr>
<tr>
<td>dl-Sotalol 100 \textmu mol/L</td>
<td>389.1±41.6*</td>
<td>538.5±38.1*</td>
<td>537.1±43.0*</td>
<td>550.8±42.9*</td>
<td>6/6*</td>
<td>5 /6*</td>
<td>3/6*</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>215.7±7.8</td>
<td>247.9±8.7</td>
<td>260.8±8.8</td>
<td>0 /6</td>
<td>0 /6</td>
<td>0 /6</td>
<td>0 /6</td>
<td></td>
</tr>
<tr>
<td>Azimilide 10 \textmu mol/L</td>
<td>594.2±43.2*</td>
<td>612.7±63.2*</td>
<td>643.8±63.1*</td>
<td>5 /6</td>
<td>1/6†</td>
<td>0/6†</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

BCL=2000 ms.

*P<0.05 vs control; †P<0.05 dl-sotalol vs azimilide.
requires more simultaneous intracellular recordings transmurally, but it is very difficult technically.

TDR seemed to play an important role in determining whether or not an EAD could propagate transmurally to induce an R-on-T extrasystole on the ECG. Although azimilide 3 to 10 μmol/L caused a more significant APD and QT prolongation than dl-sotalol, EADs induced by azimilide that originated from either epicardium or endocardium usually failed to conduct across the ventricular wall to produce R-on-T extrasystoles (Table 1). This difference in the transmural propagation of EADs between dl-sotalol and azimilide was because azimilide at higher doses decreased TDR, whereas dl-sotalol markedly increased it. TDR was 147.9±39.3 ms (n=6) in dl-sotalol 100 μmol/L versus 18.4±38.6 ms (n=6, P<0.05) in azimilide 10 μmol/L in isolated rabbit left ventricle (Figure 3B).

Clinically, the “short-long-short” sequence heralding TdP is one of the hallmarks of long-QT syndrome.1 Programmed stimulation simulating such a sequence often successfully induced EADs and associated R-on-T extrasystoles in the ventricular beats immediately after a long pause that could initiate TdP (Figure 5). An increase in QT_{end}-QT_{peak} interval in the beat immediately after a long pause was always present.

An R-on-T extrasystole may initiate the onset of TdP if TDR is great enough and ventricular mass is adequate. A marked increase in TDR not only facilitated EADs to propagate transmurally but also provided a further substrate for transmural reentry, leading to the development of TdP.3 Ventricular mass was also critical in the development of TdP. The data obtained from the small rabbit left ventricular wedge preparations (n=20), which weighed ≈33% of the entire left ventricle, have shown that frequent R-on-T extrasystoles in the presence of 50 to 100 μmol/L dl-sotalol did not induce a single episode of TdP. In contrast, R-on-T extrasystoles produced multiple episodes of TdP in 3 of 6 rabbit entire left ventricles (large preparations, Table 1). A typical episode of TdP in one of these rabbit left ventricles is shown in Figure 6.

**Discussion**

**Phase 2 EADs in Intact Ventricular Wall**

Using a recently developed arterially perfused rabbit left ventricle, we provide, for the first time, direct evidence from intracellular recordings in support of the hypothesis that EADs can be generated from the intact ventricular wall in which cells from different myocardial layers are electrically coupled. The EADs are capable of propagating transmurally under conditions of a markedly increased TDR, inducing a triggered response (R-on-T extrasystole) to initiate TdP.
Early Afterdepolarization and Torsade de Pointes

The acquired long-QT syndrome is associated with the development of TdP, a specific form of polymorphic VT, leading to syncope and sudden cardiac death. It has been appreciated that functional circus movement underlies most cases of polymorphic VT and that an extrasystole on the T wave of the preceding beat (R-on-T) is often necessary to initiate the arrhythmia. It is generally accepted that EADs may induce such a trigger to initiate TdP during QT prolongation. There are a number of pieces of indirect evidence in support of this hypothesis. In the clinic, QT prolongation in the presence of APD-prolonging agents is associated with an R-on-T phenomenon particularly during bradycardia or immediately after a long pause, ie, a so-called short-long-short sequence. R-on-T extrasystoles often herald the development of TdP. In the presence of APD-prolonging agents, fluctuation in membrane potential during the plateau phase of the action potential (phase 2 EADs), probably due to reactivation of L-type Ca or Na+-Ca exchange current, has frequently been observed in isolated sliced ventricular tissue or single myocytes. Direct evidence of EAD-induced triggered responses in in vivo conditions, however, has not yet been available. With MAP recording electrodes, phase 3 (instead of phase 2) EADs have been observed in patients with long-QT syndrome. Because the MAP electrode records electrical signals from the local ventricular surface rather than from a single myocyte, however, EAD-like activity in phases 2 and 3 may represent an artifact. Data from computer simulation and animal experiments have shown that MAP recordings within a region with marked dispersion of repolarization can manifest as EAD-like signals.

Theoretically, any transmurally conducted electrical activity during phase 2 of the action potential can manifest as an R-on-T extrasystole on the ECG. Previous studies using the canine left ventricular wedge preparation have shown that an extrastimulus using programmed electrical stimulation, which artificially served as a phase 2 EAD, had to be delivered on the downslope of the preceding T wave (R-on-T) to initiate the development of TdP during QT prolongation. Unfortunately, direct demonstration of the role of EADs in the development of polymorphic VT in the long-QT syndrome has been lacking in canine left ventricular wedge. Data obtained in the present study indicate that the difference in species is an important factor. It is well known that APDs and QT intervals in dogs are ~30% shorter than those observed in humans. In addition, maximal APD and QT prolongation in response to the I_{Kr} blocker dl-sotalol was only ~22% at a BCL=2000 ms in canine left ventricular wedge, significantly less than that (113%) observed in rabbit left ventricular preparations and in humans. A failure to record EAD in canine left ventricular wedge preparations may be partially due to the large I_{Kr} current in epicardium and endocardium, blunting QT prolongation and the development of EADs via electrotonic influence on M cells. This is supported by the finding that azimilide with combined inhibitory effects on I_{Kr} and I_{Ca}, which prolonged APD more markedly in epicardium, could produce EADs from the epicardium in canine left ventricular wedge. Conversely, EADs were frequently observed in arterially perfused rabbit left ventricle in the presence of dl-sotalol and azimilide. The difference in the generation of EADs between canine and rabbit is probably due to weaker repolarization currents, particularly I_{Kr}, in rabbit left ventricle.

EADs induced by the I_{Kr} blocker dl-sotalol usually were generated from rabbit subendocardial and endocardial layers in which APDs were preferentially prolonged. Conversely, azimilide at relatively higher concentrations (3 to 10 \mu mol/L) prolonged epicardial APD similarly to or even more than that in the subendocardial region and endocardium, inducing frequent EADs not only from subendocardium and endocardium but also from epicardium. This effect of azimilide is most likely a result of its I_{Ca} blockade. The observations that slower pacing rates and the short-long pacing sequence facilitate the generation of EADs in rabbit are consistent with clinical situations in which TdP tends to occur, indicating that EADs contribute importantly to the development of TdP.

Role of TDR in Transmural Propagation of Phase 2 EADs and the Development of TdP

Both dl-sotalol and azimilide produced frequent EADs, but transmural propagation of EADs (R-on-T extrasystoles) was observed frequently only in rabbit left ventricles pretreated with dl-sotalol, even though azimilide produced more QT-interval and APD prolongation. TDR seemed to facilitate transmural propagation of EADs. dl-Sotalol preferentially prolonged APD in subendocardial and endocardial sites, resulting in a marked increase in TDR. In contrast, azimilide produced EADs at concentrations of 3 to 10 \mu mol/L in which diminished TDR was observed due to preferential prolongation of epicardial APD. The exact transmural conduction pathway of EADs is unknown, however, on the basis of the data obtained from this study. It is interesting to note that although EAD arose in endocardium, the main vector of activation was from epicardium to endocardium, as shown in Figures 4 and 6. One possible explanation is that EAD-induced impulses from endocardium or subendocardium might reach the epicardium by traveling laterally for some distance before transmural propagation from epicardium to endocardium in the region of the ECG electrodes. Another explanation is that EADs in endocardium or subendocardium may induce an impulse in epicardium by electrotonic effects (“reflection”), which then propagates transmurally. No matter what mechanism is involved in the process from EAD to a transmurally conducted extrasystole, an increase in TDR seems to play an important role in the process. This is consistent with the clinical observation that patients with congenital long-QT syndromes have longer QT_{end}−QT_{peak} interval, an index of TDR.

An increase in TDR not only facilitates transmural propagation of EADs but also contributes importantly to the maintenance of TdP. Our data obtained from rabbit left ventricle point to transmural reentry in an adequate ventricular mass as the mechanism for the maintenance of TdP and a transmurally conducted EAD as the initiating mechanism.

Estimate of the Risk for the Development of TdP Using Isolated Rabbit Left Ventricle

Acquired long-QT syndrome and TdP induced by medical products has become an increasing concern among medical
professionals and the pharmaceutical industry. Assessment of drugs for QT prolongation and potential risk for TdP in humans is a challenging task. Our data indicate that the incidence of TdP is not related solely to the QT interval. Other factors such as TDR may also contribute. A pure \( I_{k} \) blocker seems to be more proarrhythmic than agents with combined effects on multiple currents. Therefore, the assessment of a compound for the risk of causing TdP in humans requires an evaluation to see not only whether it prolongs QT interval but also whether it increases TDR and induces EAD. The model of an isolated arterially perfused rabbit left ventricle is useful for such a purpose. In this model, transmembrane action potentials from 2 or 3 myocardial layers can be recorded simultaneously, together with an ECG. The QT interval, TDR, EADs, R-on-T extrasystoles, and TdP can be assessed simultaneously. On the basis of the effect of each compound on the QT interval, TDR, and phase 2 EAD, points are given to estimate the potential risk of TdP, as shown in Table 2. Because they are the consequence of QT prolongation and an increase in TDR, EADs and related phenomena are given a high relative weight. A score of zero indicates no predictable risk for TdP, whereas a score of 12 suggests the highest predictable risk for TdP. On the basis of the data in Table 1 and the criteria in Table 2, a score system is proposed to increase the accuracy of the assessment for the risk of proarrhythmias. For example, if a compound raises the average heart rate in humans, it should be expected to carry a lower risk no matter what its effects on the QT, TDR, and EAD are. Systematic evaluation of compounds with QT prolongation and TdP observed in the clinic is currently under way in isolated arterially perfused rabbit left ventricle.

### Acknowledgments

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### References


### TABLE 2. Score System for the Estimate of Risk of a Compound for the Development of TdP in Isolated Rabbit Left Ventricle: Points for QT Interval, TDR, and Phase 2 EAD

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<th>Point</th>
<th>QT Interval, %</th>
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<td>0</td>
<td>&lt;=10</td>
<td>&lt;=45</td>
</tr>
<tr>
<td>1</td>
<td>&lt;=10–&lt;=50</td>
<td>&gt;45–60</td>
</tr>
<tr>
<td>2</td>
<td>&gt;50–100</td>
<td>&gt;=60–90</td>
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<td>3</td>
<td>&gt;100</td>
<td>&gt;90</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Phase 2 EAD</th>
<th>No EAD</th>
<th>EAD without R-on-T</th>
<th>EAD with R-on-T</th>
<th>R-on-T with TdP</th>
</tr>
</thead>
</table>
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