Calmodulin Inhibitor W-7 Unmasks a Novel Electrocardiographic Parameter That Predicts Initiation of Torsade de Pointes

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Background—We have shown that the calmodulin inhibitor W-7 suppresses torsade de pointes (TdP) without shortening the QT interval, which is consistent with other findings that QT prolongation, per se, is insufficient to generate TdP. ECGs were analyzed from a well-characterized animal model of TdP to identify more reliable predictors of this life-threatening ventricular arrhythmia.

Methods and Results—TdP was induced using methoxamine and clofilium in 12 of 14 rabbits pretreated with vehicle control, whereas pretreatment with W-7 (50 μmol/kg), an inhibitor of the intracellular Ca2+-binding protein calmodulin, significantly suppressed TdP induction (1 of 11 rabbits with TdP, \( P < 0.001 \)). W-7 did not affect heart rate, increases in QT intervals, or dispersion compared with measurements in vehicle-treated control animals. However, a progressive and significant increase in the ratio of U-wave to T-wave amplitude (UTA) occurred before TdP onset in control animals, and this was prevented by W-7.

Conclusions—Selective suppression of TdP inducibility by W-7, without shortening the duration of cardiac repolarization, allowed identification of the UTA ratio as a new electrocardiographic index for predicting TdP onset. These findings are consistent with the idea that prolonged repolarization is not the proximate cause of arrhythmia initiation, and they suggest that an increased UTA ratio reflects activation of intracellular Ca2+-calmodulin–dependent processes that are required for triggering TdP in this model. (Circulation. 2002;105:770-774.)

Key Words: antiarrhythmia agents ■ calcium ■ electrocardiography ■ signal transduction ■ torsade de pointes

Torsade de pointes (TdP) is a form of polymorphic ventricular tachycardia usually initiated after QT prolongation and bradyarrhythmia. It is associated with sudden cardiac death both in the congenital form1,2 and in the acquired long-QT syndrome, which often is provoked by action potential–prolonging drugs.3,4 TdP remains an important clinical challenge because of increasing recognition of congenital long-QT syndromes and the ongoing risk posed to millions of patients taking QT interval–prolonging drugs. Identification of the molecular triggers for TdP is an area of active inquiry, and a growing body of work has highlighted the importance of intracellular calcium signaling for induction of TdP.5-11 Increased calcium activates many intracellular targets, including the calcium-binding protein calmodulin (CaM), and the CaM inhibitor W-7 was recently reported to suppress TdP induction without shortening the QT duration.9 This observation suggested the possibility that W-7 could be used as a probe to test the hypothesis that electrocardiographic parameters linked to CaM-dependent cellular signaling could predict the development of TdP.

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See p 675

Presently available electrocardiographic parameters are unsatisfactory for predicting TdP onset, and improved TdP predictors are needed for prevention and timely treatment of this life-threatening arrhythmia. QT dispersion (QTd) is one electrocardiographic parameter that has been reported to reflect heterogeneity of ventricular repolarization, and increased QTd is associated with malignant ventricular arrhythmias in patients with structural heart disease,12 excessive QT prolongation from antiarrhythmic drugs,13 and in the congenital long-QT syndromes.14,15 However, the independent prognostic significance of QTd is uncertain. Furthermore, all ECG duration measurements are complicated by the difficulty in precisely and accurately determining the end of the T or U wave.16,17 The previously reported finding that W-7 could suppress TdP without shortening the QT indicated that QT prolongation is not the proximate cause of TdP. However, W-7’s effects on other electrocardiographic repolarization parameters, including QTd, are unknown. This study was
undertaken to test the hypothesis that electrocardiographic parameters predictive of TdP initiation and reflecting the CaM-activated molecular machinery for triggering TdP are revealed by W-7.

Methods

Rabbit Arrhythmia Model

The in vivo rabbit model of TdP was adapted from Carlsson et al. with minor modifications as previously described. Male New Zealand rabbits (2.5 to 3.5 kg) (Myrtle’s Rabbitry, Thompson Station, Tenn) were anesthetized with ketamine (35 mg/kg IM) and xylazine (5 mg/kg IM). Supplemental xylazine (1 mg/kg IM) and ketamine (15 mg/kg IM) were given 15 minutes after the initial doses to maintain adequate anesthesia (loss of withdrawal reflex) throughout the experiment. Rabbıts were mechanically ventilated with room air (Harvard Rodent Ventilator), and arterial blood pressure was continuously monitored via a femoral artery cannula. There were no significant differences in systolic or diastolic arterial pressure in W-7- or vehicle-treated groups, similar to a previous report. Methoxamine (70 nmol/kg per minute) in vehicle solution (5% dextrose, 20 mL IV total) was infused for controls; W-7 (50 μg/kg in 20 mL IV total; Biomol) was infused for the experimental group during the first 10 minutes (Figure 1). Thereafter, clofilium (100 nmol/kg per minute) and methoxamine were infused together for 30 minutes or until TdP induction occurred (Figure 1). After the study, animals were euthanized with pentobarbital (50 mg/kg IV) and KCl (1 mL, 3 mol/L IV). All procedures were approved by the Vanderbilt University Animal Care Committee.

ECG Recording

Standard surface ECG limb leads (I, II, III, aVF, aVL, aVR), a midsternal chest lead (V1), and a midaxillary chest lead (V6) were monitored continuously and digitally acquired (499-Hz sampling) with a personal computer using a 12-lead ECG amplifier and Ponemah software (both from Gould Instrument Systems). Records from 4 control and 2 W-7 experiments were deleted before complete ECG interval analysis because of difficulties with an early version of this software. TdP was defined as 6 consecutive beats of polymorphic ventricular tachycardia (Figure 2).

ECG Interval Measurements

QT measurements were recorded from the onset of the QRS complex to the return of the T wave to the isoelectric line but also included the U wave when present at 25% of the T-wave amplitude. QT intervals were measured for 3 consecutive sinus beats at 6 consecutive 4-minute intervals and at 30 minutes, or until the occurrence of bigeminy or sustained TdP. The QT was corrected for variation in heart rate (QTc) using the following formula developed for rabbits: QTc = QT / √(RR). The QTd was defined as the longest QT interval minus the shortest QT interval (also including the U wave when present, as above) among 8 leads. The dispersion values were calculated for each beat separately, and QTd is the mean for 3 consecutive sinus beats analyzed. All measurements were performed manually with an online electronic caliper at 50-mm/s sweep speed to improve resolution of T- and U-wave terminal segments.

RR Interval

The RR interval was measured from the onset of consecutive QRS complexes.

T- and U-Wave Analysis

The amplitudes of the T and U waves were analyzed in the lead with the highest U-wave amplitude and the clearest distinction between the T and U waves, according to a previously published method with minor modifications. Development of U waves was judged independently by 2 observers who were blinded to treatment status 30 seconds before the first premature ventricular contraction (PVC) or at the end of the experiment (Figure 1), whichever came first. A distinct U wave had to be visualized in at least 2 limb leads and was graded from 0 to 2. A grade of 0 meant no U wave was present. Grade 1 indicated 2 distinct components of repolarization were identified, as defined by 2 tangent lines, each with a slope equal to 0, where the repolarization components were not separated by a clear nadir point. Grade 2 indicated that distinct T- and U-wave forms were present and separated by a nadir point. The ratio of U-wave to T-wave amplitude (UTA) was analyzed, by design, if the 2 observers blinded to the treatment status gave a combined score ≥3 but there were no interobserver disagreements.

Chemicals

Chemicals were obtained from Sigma unless otherwise noted. Solutions were prepared fresh daily from concentrated stock solutions.

Statistical Analysis

Mean±SEM was calculated for continuous variables, and absolute and relative frequencies were measured for discrete variables. Continuous variables were compared between groups with Student’s t test or 1-way analysis of variance (ANOVA), and post hoc comparisons were performed with Bonferroni-corrected t tests, as appropriate. Categorical variables were compared with Fisher’s exact test. The null hypothesis was rejected for P<0.05.

Results

W-7 Suppresses TdP Induction

In control animals treated with methoxamine and clofilium, a consistent evolutionary pattern of changes was observed. Bradycardia and QT prolongation were followed by fractionation of the T wave into 2 peaks (T and U), with a progressive increase in U-wave amplitude occurring immediately before
Tdp initiation (Figure 2). Tdp induction was significantly suppressed \( (P<0.001) \) in rabbits treated with W-7 (1 of 11 inducible) compared with vehicle control (12 of 14 inducible). The presence of PVCs also was diminished significantly \( (P<0.001) \) by W-7 (2 of 11 with PVCs) compared with animals treated with vehicle control (14 of 14 with PVCs, \( P<0.001 \)).

**QT and Heart Rate Are Not Affected by W-7**

Bradyarrhythmia and QT prolongation are associated with Tdp development in patients\(^{12} \) and in this rabbit model.\(^{18} \) Marked heart rate slowing and QT and QTc interval prolongation followed treatment with methoxamine and clofilium (Figure 3), and these electrocardiographic parameters were similar in control and W-7–treated animals. Thus, suppression of TaP and PVCs by W-7 was not caused by effects on QT or QTc intervals, or heart rate, suggesting that cellular events reflected by these electrocardiographic parameters are insufficient for development of Tdp.

**W-7 Has No Effect on QT Dispersion**

QT dispersion (QTd) may predict the arrhythmogenic potential of patients in whom cardiac repolarization is altered by drugs,\(^{13} \) structural heart disease,\(^{12} \) or the congenital long-QT syndromes.\(^{15} \) QTd increased equally in W-7– and vehicle-treated animals (Figure 4). However, QTd increases did not reach statistical significance in either control \( (P=0.27) \) or W-7–treated \( (P=0.52) \) animals. These findings show that suppression of Tdp by W-7 occurs in the absence of increases in QTd, suggesting that QTd does not reflect electrophysiological mechanisms fundamental to Tdp in this model.

**UTA Ratio Increases Predict Tdp Initiation and Are Prevented by W-7**

The QT split into 2 peaks (Figure 5), and the second peak (ie, the U wave) increased significantly in amplitude (Figure 6) immediately before the first PVC. U waves were present in 7 of 9 rabbits before Tdp onset but were present in only 3 of 12 rabbits without Tdp \( (P=0.03) \), suggesting that the presence of a U wave might reflect activation of cellular processes driven by Ca\(^{2+} / \)CaM–dependent signaling and favoring Tdp onset. This hypothesis was supported by the finding that U waves were only present in 2 of 11 rabbits treated with W-7 compared with 8 of 10 rabbits treated with control vehicle.

**Figure 3.** W-7 does not prevent heart rate slowing or QT prolongation by methoxamine and clofilium. A, QT-interval durations increase significantly for both control \( (P=0.001; \) open circles) and W-7–treated rabbits \( (P<0.001; \) filled circles). B, Heart rate is slowed significantly for both control \( (P=0.001) \) and W-7–treated \( (P<0.001) \) rabbits. No significant differences were present between control and W-7 groups at any time point in panels A or B. C, Rate-corrected QT (QTc) also increased significantly for control \( (P=0.028) \) and W-7–treated \( (P<0.001) \) rabbits. Numerals at the top indicate the number of animals measured for each data point; controls are the top numerals and W-7–treated animals are the bottom numerals.

**Figure 4.** QT-interval dispersion was not different in control or W-7–treated rabbits. QT dispersion did not increase significantly compared with baseline \( (0 \text{ min}) \) in control \( (P=0.27) \) or W-7–treated rabbits \( (P=0.52) \). No significant differences were present between control and W-7–treated animals at any measured time point. Numerals at the top indicate the number of control (upper) and W-7–treated (lower) rabbits. Data from control rabbits are indicated by open circles, and W-7–treated animals are shown by the filled circles.

**Figure 5.** U-wave amplitude increases are suppressed by W-7. Rows A through D show ECG tracings and evolutionary changes from vehicle-control (A and B) and W-7–treated (C and D) rabbits at baseline, after 12 min, and at the first PVC or after 30 min of clofilium infusion (Figure 1). Giant U waves (marked by arrowheads) are evident before the PVC only in control rabbits. In contrast, W-7 suppresses giant U-wave development. RR intervals are equalized to improve comparison of T-wave morphology, and horizontal bars are 200 ms throughout.
changes in ventricular repolarization with various disease processes influence QTd. The finding that TdP increases are associated with sudden cardiac death and TdP in some reports, and it remains uncertain which electrophysiological processes influence QTd. The finding that the increase in QTd before TdP was not significant is thus in line with some previous findings but not others. The fact that QTd increases only variably predict arrhythmia initiation is consistent with the possibility that different mechanisms may underlie TdP in various models and clinical settings. T-wave vector loops may prove to be a useful electrocardiographic tool for linking changes in ventricular repolarization with various disease states. However, presently available methods for T-wave vector loop acquisition and processing are cumbersome, and there is a paucity of data about underlying molecular and cellular mechanisms. Our finding that TdP can be suppressed without QT shortening by a CaM inhibitor motivated the present investigation for a novel electrocardiographic index linked to CaM-dependent cellular signaling. The UTA ratio is hypothesized to account for the U wave and provide the functional substrate for maintenance of TdP. Excessive prolongation of cardiac repolarization also increases intracellular Ca2+, and activates CaM and Ca2+/CaM–dependent protein kinase (CaMK). Although CaM can activate diverse signaling molecules, recent evidence has specifically linked activation of CaMK to early and delayed afterdepolarizations—both of which are hypothesized to trigger PVCs for TdP. CaMK is thought to stimulate early afterdepolarizations by increasing L-type Ca2+ channel activity, whereas other cellular studies have linked delayed afterdepolarizations to CaMK activation of inward Na+/Ca2+ exchanger current. Afterdepolarizations most frequently arise in the M-cell layer and are thought to further increase the intramyocardial repolarization gradient, giving rise to giant U waves. The finding that the UTA ratio was significantly suppressed by W-7 supports the novel hypothesis that U waves are critically dependent on afterdepolarizations that are activated by Ca2+/CaM.

### Study Limitations
W-7 is an effective CaM-inhibitory agent, but chemically related agents are also direct L-type Ca2+ current antagonists. Thus, observed effects on TdP could be by direct action at ion channel proteins, in addition to CaM inhibition. However, the present study and previous findings showed that the concentration of W-7 used here does not reduce blood pressure, slow heart rate, or change the QT interval, suggesting that significant direct Ca2+ channel antagonist action does not occur in vivo under our conditions. The protein kinase A inhibitory agent H-8 has recently been shown to suppress TdP, but only with concomitant QT shortening, suggesting that separation of marked QT prolongation from TdP inducibility may be unique to CaM-inhibitory agents. Although the best evidence suggests that W-7’s effects are likely due to inhibition of Ca2+/CaM–dependent kinase II, a more selective inhibitory agent will be required to definitively determine the molecular mechanism for electrocardiographic changes in TdP.

### Molecular Mechanism for Electrocardiographic Changes in TdP
The present findings show that excessive prolongation of cardiac repolarization alone does not explain the mechanism for TdP. Action-potential prolongation by class III antiarrhythmic agents is disproportionately prolonged in M cells, and the repolarization gradient between M cells and more rapidly repolarizing cells in the epicardium and endocardium is hypothesized to account for the U wave and provide the functional substrate for maintenance of TdP. Excessive prolongation of cardiac repolarization also increases intracellular Ca2+, and activates CaM and Ca2+/CaM–dependent protein kinase (CaMK). Although CaM can activate diverse signaling molecules, recent evidence has specifically linked activation of CaMK to early and delayed afterdepolarizations—both of which are hypothesized to trigger PVCs for TdP. CaMK is thought to stimulate early afterdepolarizations by increasing L-type Ca2+ channel activity, whereas other cellular studies have linked delayed afterdepolarizations to CaMK activation of inward Na+/Ca2+ exchanger current. Afterdepolarizations most frequently arise in the M-cell layer and are thought to further increase the intramyocardial repolarization gradient, giving rise to giant U waves. The finding that the UTA ratio was significantly suppressed by W-7 supports the novel hypothesis that U waves are critically dependent on afterdepolarizations that are activated by Ca2+/CaM.
specific CaM-activated molecular target responsible for U-wave amplitude increases and TdP.

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References
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