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

T. David Gbadebo, MD*; Robert W. Trimble, BS*; Michelle S.C. Khoo, MB, BCh; Joel Temple, MD; Dan M. Roden, MD; Mark E. Anderson, MD, PhD

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 Ca^{2+}-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 Ca^{2+}/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 bradycardia. It is associated with sudden cardiac death both in the congenital form and in the acquired long-QT syndrome, which often is provoked by action potential–prolonging drugs. 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. 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. 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.
The dispersion values were recorded from the onset of the QRS complex (vertical) and 200 ms (horizontal) throughout.

**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) 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. Rabbits 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 control and methoxamine group, similar to a previous report. Methoxamine (70 nmol/kg per minute) was infused intravenously. W-7 or D5W was stopped after 10 min and clofilium infusion was started. Clofilium and methoxamine were continued for 30 min or until sustained TdP, whichever occurred first.

 undertook 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.

ECG Recording

Standard surface ECG limb leads (I, II, III, aVF, aVL, aVR), a midternal 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 increase in U-wave amplitude occurring immediately before 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
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=0.02) and W-7–treated rabbits (P<0.001). * indicates significant changes compared with baseline (0 min) for individual points; controls are the top numerals and W-7–treated animals are the bottom numerals.

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 by W-7 (2 of 11 with PVCs) compared with animals treated with control vehicle (14 of 14 with PVCs, P<0.001).

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 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 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 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 offers important advantages over previously recognized electrocardiographic parameters, including a minimal requirement for data processing and independence from measuring the end of the T wave, which suggest the UTA ratio could be incorporated into algorithms for guiding drug or pacing therapies for TdP.

Figure 6. The UTA ratio increases significantly before PVC initiation. A, U-wave amplitude increases significantly before the first PVC (P=0.021). In contrast, the T-wave amplitude does not increase significantly (P=0.971) compared with baseline. The first time point (abscissa) is 12 min after initiating the experimental protocol (Figure 1), and all data points after the break are marked by time before the first PVC for both panels. The numerals in the upper panel indicate the number of animals studied for each data point and apply to both panels. B, UTA ratio increases significantly compared with baseline (P=0.008) before the first PVC in animals that developed TdP. Significant increases compared with the first (12 min) data point.

(P=0.009). The UTA ratio was formulated to normalize U-wave amplitude changes to the T-wave amplitude, thereby minimizing potential differences in recordings between individual rabbits. The UTA ratio increased significantly (P=0.008) immediately before PVC initiation in animals that developed TdP (Figure 6B), similar to changes seen in the U-wave amplitude (Figure 6A).

Discussion

Electrocardiographic Parameters Associated With TdP

The QT duration and QTd are electrocardiographic parameters used to assess proarrhythmic potential of drugs, congenital long-QT syndromes, and heart failure in patients and in animal models. The QT duration is advantageous because it can be performed rapidly, but its utility is reduced by technical difficulties with defining the end of the T wave and by the fact that a threshold value for QT prolongation that reliably predicts arrhythmia remains undefined. QTd 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 processes influence QTd. 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 Ca\(^{2+}\), and activates CaM and Ca\(^{2+}/\text{CaM}\)–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 triggers for PVCs and TdP. CaMK is thought to stimulate early afterdepolarizations by increasing L-type Ca\(^{2+}\) channel activity, whereas other cellular studies have linked delayed afterdepolarizations to CaMK activation of inward Na\(^+/\text{Ca}^{2+}\) 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 Ca\(^{2+}/\text{CaM}\).

Study Limitations

W-7 is an effective CaM-inhibitory agent, but chemically related agents are also direct L-type Ca\(^{2+}\) 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 Ca\(^{2+}\) 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 Ca\(^{2+}/\text{CaM}\)–dependent kinase II, a more selective inhibitory agent will be required to definitively determine the
specific CaM-activated molecular target responsible for U-wave amplitude increases and TdP.

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References
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