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 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.

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, excessive QT prolongation from antiarrhythmic drugs, and in the congenital long-QT syndromes. 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. 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...
untaken 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); Suplemental 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 a premature beat and TdP were continued for 30 min or until sustained TdP, whichever occurred first.

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 1. Schematic depiction of the experimental protocol. After instrumentation and a 10-min stabilization period, W-7 or 5% dextrose solution (D5W) vehicle control and methoxamine were 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.

Figure 2. Representative ECG tracings in a vehicle-treated control animal show evolution of the U-wave amplitude before initiation of TdP. A, Baseline ECG tracing (lead III) before initiating infusion of control vehicle. B, ECG tracing 12 min after initiating the experimental protocol (Figure 1) shows emergence of a U wave (marked by arrowhead for the first beat in this and subsequent panels) seen as a secondary deflection separated from the T wave by a clear nadir point. C and D show progressive evolution of the U-wave amplitude 2 min (C) and 10 s (D) before TdP initiation. E, U-wave amplitude increases further and is associated with premature beats and TdP. Scale bar is 10 mV (vertical) and 200 ms (horizontal) throughout.

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.

Chemicals

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

Figure 2.

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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 (P<0.001) rabbits. 

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.

The 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.

QT and Heart Rate Are Not Affected by W-7
Bradyarrhythmia and QT prolongation are associated with TdP development in patients and in this rabbit model. 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, structural heart disease, or the congenital long-QT syndromes. 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

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.
increases are associated with sudden cardiac death and TdP in various models and clinical settings. T-wave vector loops have been investigated as a measure of early afterdepolarizations and of the possibility that different mechanisms may underlie TdP in various processes. However, prior to the present investigation, the mechanisms underlying TdP were not well understood. The present study investigated changes in ventricular repolarization with the goal of developing a novel electrocardiographic index for guiding drug or pacing therapies for TdP.

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. 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 to inhibition of Ca2+/CaM–dependent kinase II, a more selective inhibitory agent will be required to definitively determine the

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).

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 triggers for PVCs and 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
specific CaM-activated molecular target responsible for U-wave amplitude increases and TdP.

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