Systemic Administration of Calmodulin Antagonist W-7 or Protein Kinase A Inhibitor H-8 Prevents Torsade de Pointes in Rabbits

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Background—The ventricular arrhythmia torsade de pointes (TdP) occurs after QT interval prolongation and is associated with sudden cardiac death. The afterdepolarizations that initiate TdP are facilitated by protein kinase A and the multifunctional Ca\(^{2+}\)/calmodulin-dependent protein kinase II (CaM kinase).

Methods and Results—In this study, we evaluated the feasibility of suppression of TdP through systemic therapy with kinase inhibitory agents in an established animal model. Under control conditions, TdP was inducible in 6 of 8 rabbits. CaM kinase blockade with the calmodulin antagonist W-7 reduced TdP in a dose-dependent fashion (4 of 7 inducible at 25 \(\mu\)mol/kg and 1 of 7 inducible at 50 \(\mu\)mol/kg). Increased intracellular Ca\(^{2+}\) has been implicated in the genesis of afterdepolarizations, but pretreatment with high-dose W-7 did not prevent TdP in response to the L-type Ca\(^{2+}\) channel agonist BAY K 8644 (300 nmol/kg), suggesting that CaM kinase–independent activation of L-type Ca\(^{2+}\) current was not affected by W-7. Compared with control animals, W-7 reduced TdP inducibility without shortening the QT interval, increasing heart rate, or reducing the blood pressure. The protein kinase A antagonist H-8 also caused a dose-dependent reduction in TdP inducibility (5 of 6 at 1 \(\mu\)mol/kg, 4 of 6 at 5 \(\mu\)mol/kg, and 0 of 6 at 10 \(\mu\)mol/kg), but unlike W-7, H-8 did so by shortening the QT interval.

Conclusions—These findings show that the acute systemic application of W-7 and H-8 is hemodynamically tolerated and indicate that kinase inhibition may be a viable antiarrhythmic strategy. (Circulation. 1999;100:2437-2442.)

Key Words: torsade de pointes ■ long-QT syndrome ■ calmodulin kinase

Drugs that prolong action potential (AP) repolarization can be antiarrhythmic through the lengthening of tissue refractoriness or the elimination of an excitatory gap, thereby extinguishing reentry.\(^1\) AP prolongation also has proarrhythmic consequences by causing afterdepolarizations that trigger torsade de pointes (TdP) and that are associated with sudden death. An agent that could preserve the beneficial effects of AP prolongation without inducing afterdepolarizations and TdP thus is an important goal for antiarrhythmic drug development. Protein kinase A (PKA), Ca\(^{2+}\)/calmodulin-independent protein kinase II (CaM kinase), and increased intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) have been implicated as causes of afterdepolarizations in isolated cardiac myocytes\(^3–6\) and isolated Langendorff-perfused heart models.\(^7\) PKA and CaM kinase both likely enhance the probability of afterdepolarizations by increasing [Ca\(^{2+}\)]\(_i\), and the systemic administration of the sarcoplasmic reticulum (SR) Ca\(^{2+}\) uptake and release antagonists flunarizine\(^8\) and ryanodine\(^9\) prevents TdP. PKA and CaM kinase both act at L-type Ca\(^{2+}\) channels\(^10,11\) and SR Ca\(^{2+}\) stores\(^12–14\) to increase [Ca\(^{2+}\)]\(_i\).

Afterdepolarizations are oscillations in cell membrane potential\(^15,16\) that are often associated with increased SR Ca\(^{2+}\) release and are suppressed by CaM kinase inhibition in isolated Langendorff-perfused hearts\(^7\) and cardiac myocytes.\(^3\) Recently, AP prolongation in response to the class III antiarrhythmic drug clofilium was shown to increase CaM kinase activity\(^7\) when afterdepolarizations were induced. The application of a CaM kinase inhibitor prevented the increase in CaM kinase activity without shortening AP duration.\(^7\) Afterdepolarizations are also associated with conditions favoring increased PKA activity,\(^4–6\) and \(\beta\)-adrenergic receptor antagonists likely exert an antiarrhythmic action in long QT–related arrhythmias by decreasing PKA activity. Because of the known link among PKA, CaM kinase, and triggered arrhythmias, it is likely that inhibition of these kinases may prevent TdP. However, even the acute hemodynamic feasibility of the treatment of arrhythmias with systemically administered kinase inhibitory agents is unknown.

An ideal therapeutic kinase inhibitory agent would be systemically bioavailable, cell membrane permeant, highly specific, and potent and would not be associated with side effects. At the present, no such inhibitors are thought to exist. W-7 [N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide]...
is a water-soluble, cell membrane-permeant competitive antagonist of calmodulin.\textsuperscript{17} W-7 IC\textsubscript{50} values for calmodulin-dependent enzymes range from \textapprox{}25 to 50 \textmu{}mol/L. Calmodulin is an ubiquitous intracellular Ca\textsuperscript{2+}-binding protein with a bilobed tertiary structure containing 4 Ca\textsuperscript{2+}-binding EF hand domains.\textsuperscript{18} Calmodulin undergoes conformational changes induced by Ca\textsuperscript{2+} binding that exposes the activation site for calmodulin-dependent enzymes such as CaM kinase. W-7 inhibits activation of calmodulin-dependent enzymes by blocking access to this hydrophobic activation site (K\textsubscript{i} \approx{} 12 \textmu{}mol/L).\textsuperscript{18} W-7 is a less potent PKA or protein kinase C (PKC) antagonist (K\textsubscript{i} \approx{} 110 \textmu{}mol/L).\textsuperscript{19,20} H-8 [N-[2-methylaminoethyl]-5-isoquinolinesulfonamide] is a water-soluble, cell membrane-permeant derivative of W-7 with enhanced inhibitory activity against a variety of protein kinases, including protein kinase G (K\textsubscript{i} = 0.5 \textmu{}mol/L), PKA (K\textsubscript{i} = 1.2 \textmu{}mol/L), and PKC (K\textsubscript{i} = 15 \textmu{}mol/L), but without appreciable activity against CaM kinase.\textsuperscript{17} These agents were chosen for systemic use because of their high water solubility and cell membrane permeability and their demonstrated inhibitory efficacy in a wide range of mammalian cells.\textsuperscript{17} In this study, the effects of these 2 kinase inhibitors, administered systemically, were tested in an established animal model of TdP.

**Methods**

**Rabbit Arrhythmia Model**

The in vivo rabbit model of TdP was implemented as described by Carlson et al\textsuperscript{21} with minor modifications. In brief, male New Zealand rabbits (weight 2.5 to 3.0 kg) were initially anesthetized with 35 mg/kg ketamine IM and 5 mg/kg xylazine IM. Supplemental xylazine (1 mg/kg IM) and ketamine (15 mg/kg IM) were administered 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 the adjustment of respiratory parameters was guided by arterial blood gases (PO\textsubscript{2} 84 to 101 mm Hg, PCO\textsubscript{2} 33 \textpm{} 0.6 mm Hg, pH 7.44 \pm{} 0.03). Arterial blood pressure was continuously monitored with a femoral artery cannula. A minimum 10-minute stabilization period was used before any further intervention. Methoxamine (70 nmol \cdot kg\textsuperscript{-1} \cdot min\textsuperscript{-1} IV) was then infused for 10 minutes before starting clofilium (100 nmol \cdot kg\textsuperscript{-1} \cdot min\textsuperscript{-1} IV), after which both agents were continued simultaneously for 30 minutes or until TdP induction. Animals were euthanized with 50 mg/kg pentobarbital IV after the study. All procedures performed in the present study were approved by the Vanderbilt University Animal Care Committee.

**Systemic Application of Kinase Inhibitors**

Control animals were infused with 5\% dextrose solution (20 mL total IV) at the same time as methoxamine infusion. The experimental group animals were infused with 5\% dextrose solution (20 mL total IV) containing the indicated concentration of W-7 or H-8 (both from BIOMOL Research Laboratories) during methoxamine infusion. A separate group of 4 rabbits were pretreated with 50 \textmu{}mol/kg W-7 and observed for 25 minutes without TdP after the methoxamine and clofilium infusion, as described. Then, 1 mL of the vehicle for the L-type Ca\textsuperscript{2+} channel agonist BAY K 8644 was administered, which did not elicit TdP or any hemodynamic changes. BAY K 8644 (300 nmol/kg IV bolus in 1 mL polyethylene glycol/ethanol/saline [5:5:90 vol/vol]; BIOMOL Research Laboratories) was then administered over 30 seconds, and the animal was observed for 5 minutes.

**ECG Recording**

Standard surface ECG limb leads (I, II, III, aVF, aVL, and aVR) and a midchest lead positioned at the sternal notch (V) were continuously monitored and recorded at a paper speed of 100 mm/s (Electronics for Medicine, Honeywell Inc). ECG intervals were analyzed with a digitizing tablet (Summagraphics Corp) interfaced to a microcomputer. A 2-channel ECG was continuously recorded on audiotape and analyzed with the use of an ambulatory ECG monitoring system (Rozinn Electronics Inc).

TdP TdP was defined as \geq{}6 consecutive beats of polymorphic ventricular tachycardia.

**ECG Interval Measurements**

ECG intervals were measured as the average from 3 consecutive beats with a single lead providing the clearest end of the QT interval (usually lead II or III).

**QT Interval**

QT interval measurements were recorded from the onset of the QRS complex to the return of the T wave to the isoelectric line (Figure 1). When present at \geq{}25\% of the amplitude of the T wave, the U wave was included in the QT duration measurement.\textsuperscript{22}

**Corrected QT Interval**

The QT interval was corrected (QT\textsubscript{c}) according to the method of Carlson et al\textsuperscript{23} for rabbits with the formula QT\textsubscript{c} = QT - 0.175(RR - 300).

**RR Interval**

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

**Chemicals**

All chemicals were obtained from Sigma Chemical Co, unless otherwise noted, and solutions were prepared fresh daily from concentrated stock solutions.
**Statistical Analysis**

Mean±SD values was calculated for continuous variables, and absolute and relative frequencies were measured for discrete variables. Continuous variables were compared between groups with the use of Student’s *t* test or 1-way ANOVA as appropriate, and categorical variables were compared with the use of Fisher’s exact test. Values of *P*<0.05 were considered statistically significant.

**Results**

**Induction of TdP**

After treatment with methoxamine and clofilium, a consistent sequential pattern of bradycardia, QT interval prolongation, and isolated premature ventricular contractions (PVCs) leading to long-short coupling intervals preceded TdP initiation (Figure 1A). The time to the first PVC was significantly increased in W-7–treated animals (19.0±8.0 minutes) compared with control (7.7±5.9 minutes, *P*, 0.01) or H-8–treated (5.7±4.4 minutes, *P*, 0.001) animals. TdP was induced in 6 of 8 rabbits under control conditions (Figure 2).

**Prevention of TdP by W-7 and H-8**

Both W-7 and H-8 prevented TdP inducibility in a dose-dependent manner (Figure 2), consistent with the reported roles of CaM kinase and PKA in the facilitation of afterdepolarizations. TdP suppression was significant at 50 μmol/kg for W-7 and at 10 μmol/kg for H-8. TdP induction in W-7–pretreated animals tended to occur later after the infusion of methoxamine and clofilium than in controls. TdP was induced with the L-type Ca2+ current agonist BAY K 8644 after W-7 (50 μmol/kg) pretreatment (n=4), suggesting that these animals remained capable of developing TdP by a calmodulin- and CaM kinase–independent pathway (Figure 3).

**QT Interval Not Affected by W-7**

QT interval prolongation favors induction of TdP, so suppression of TdP by kinase inhibitory agents could simply reflect QT interval shortening.24 Marked QT and QTc interval prolongation occurred after treatment with methoxamine and clofilium, and this prolongation was not affected by pretreatment with W-7 (Figures 1B and 4). Treatment with H-8 (10 μmol/kg) resulted in significant QT and QTc interval shortening (Figure 4). Thus, suppression of TdP by W-7, but not by H-8, was independent of QT interval prolongation.

**Prevention of TdP by W-7 Not Due to Increase in Heart Rate**

Because inducibility of TdP is known to be favored at low heart rates,25 one possible mechanism for TdP suppression could be an increase in heart rate. The heart rate slowed during the course of methoxamine and clofilium infusion but was not significantly different from control rates in W-7 (50 μmol/kg)–treated animals (Figure 5). In contrast, H-8 (10 μmol/kg) increased heart rate at all time points (Figure 5). The suppression of TdP by W-7, but not by H-8, was independent of an effect on heart rate.

**Kinase Antagonist Therapy Was Acutely Hemodynamically Tolerated**

The infusion of W-7 or H-8 at levels adequate to suppress TdP was not associated with a decrease in systolic (Figure 6A) or diastolic (Figure 6B) blood pressure. On the contrary, W-7–treated animals had a tendency to increase both systolic and diastolic blood pressure. Thus, the systemic administration of these kinase inhibitory agents was acutely hemodynamically tolerated in this animal model.

**Discussion**

**Serine/Threonine Kinases in Cardiovascular System**

Because serine/threonine kinases are ubiquitous and regulate a diverse array of target proteins in the cardiovascular system, the consequences of the use of systemic kinase inhibitory agents are unknown. In the cardiovascular system, PKA and
PKC are both known to affect heart rate, 26,27 and PKA, 28,29 protein kinase G, 28,34 and CaM kinase 10,13,14,35–37 all participate in the regulation of smooth and cardiac muscle \([\text{Ca}^{2+}]_i\). Thus, the acute hemodynamic response to the systemic application of these inhibitory agents could be an important limitation in the development of novel approaches to antiarrhythmic therapy with the use of serine/threonine kinase inhibitory agents. On the other hand, the inhibition of the cardiac cell membrane ionic currents and exchangers responsible for afterdepolarizations may be accomplished with sufficiently low concentrations of kinase inhibitory agents to avoid systemic toxicities. New approaches to drug development through the use of combinatorial chemistry may lead to agents with increased potency and specificity compared with currently available agents.

Role of Serine/Threonine Kinases in Arrhythmias

Both PKA and CaM kinase are thought to have proarrhythmic actions due to enhancement of L-type \(\text{Ca}^{2+}\) current. 3–5,10 PKA has long been an “indirect” antiarrhythmic drug target because clinically available \(\beta\)-adrenergic receptor antagonists prevent \(\beta\)-agonist mediated increases in PKA activity. These agents have been shown to reduce sudden cardiac death 39 and are used in patients with long QT syndromes. 25 In contrast to PKA, CaM kinase activity is generally not attributable to the activation of a single receptor type but rather increases in response to elevated \([\text{Ca}^{2+}]_i\).40 CaM kinase activity increases during AP prolongation and afterdepolarizations, and CaM kinase inhibition prevents afterdepolarizations without shortening AP duration in isolated hearts.7 Although cell membrane permeant kinase inhibitors have long been available as pharmacological and research tools,17 the ubiquitous nature of these kinases has perhaps been viewed as a potential obstacle to systemic kinase inhibition for therapy of cardiac arrhythmias. The findings presented here suggest that at least for acute administration, 2 such agents are hemodynamically tolerated at concentrations effective for the suppression of arrhythmias.

Kinase Inhibitory Agents

Although a role of CaM kinase in the facilitation of afterdepolarizations in isolated cells has been inferred with the use of highly specific inhibitory peptides, 2,3 no cell membrane–permeable agents with similar specificity adequate for systemic administration presently exist. Both W-7 and H-8 can

Figure 4. QT intervals in control and H-8– and W-7–pretreated rabbits. A, QT intervals uncorrected for heart rate in vehicle-pretreated control rabbits and rabbits pretreated with W-7 (50 \(\mu\)mol/kg) or H-8 (10 \(\mu\)mol/kg) at baseline (time 0) and after infusion of methoxamine and clofilium (initiation and duration of infusion are indicated by arrows). Top, Total number of animals in each group for each measurement, in this and all subsequent figures. Decrease in these numbers over time indicates TdP induction in control group. Maximum (Max) QT interval measured within 25 minutes of clofilium and methoxamine infusion is also plotted for each group. B, Rate-corrected QT intervals (see “Methods”) in same rabbits represented in A. *\(P<0.05\), ***\(P<0.001\) compared with control. Symbols for control and treatment groups are the same for both panels.

Figure 5. Heart rate, plotted as RR interval, in control and H-8 (10 \(\mu\)mol/kg)– and W-7 (50 \(\mu\)mol/kg)–pretreated rabbits. Data are displayed in same format as in Figure 4A. *\(P<0.05\), **\(P<0.01\), ***\(P<0.001\) compared with control.

Figure 6. Systolic (A) and diastolic (B) arterial blood pressure in control and H-8 (10 \(\mu\)mol/kg)– and W-7 (50 \(\mu\)mol/kg)–pretreated rabbits. Data are displayed in same format as in Figure 4. Symbols for control and treatment groups are the same for A and B. *\(P<0.05\) compared with control.
Prevention of TdP In Vivo
The suppression of TdP by both W-7 and H-8 occurred in a dose-dependent manner. H-8 did not suppress TdP at concentrations predicted to be selective for PKA inhibition, although the effective intracellular concentration is unknown. At higher concentrations, compatible with PKA and PKC inhibition, H-8 was effective in the suppression of TdP. W-7 at concentrations predicted to inhibit calmodulin-dependent processes, including those mediated by CaM kinase, was also effective in the suppression of TdP. The findings that PVC onset was delayed by W-7 suggests that CaM kinase inhibition reduced the probability of a triggering event (ie, an afterdepolarization). Our findings do not provide information regarding possible affects of kinase inhibitors on the substrate for arrhythmia maintenance (eg, dispersion of repolarization). It will be important to better define the mechanisms of action of kinase inhibitory agents in TdP prevention in future studies through the measurement of afterdepolarizations and QT interval dispersion. Although the rabbit TdP model of the present study is highly reproducible and widely used, studies in other models, and ultimately in humans, will be required to determine the potential clinical use of kinase inhibition as a therapy for arrhythmias.

Proarrhythmia and QT Interval Prolongation
The inhibition of TdP by W-7 was different from that seen after treatment with H-8. The most striking difference was that W-7 did not result in a decrease in QT interval, whereas H-8 treatment significantly shortened the QT interval. QT interval shortening suggests that effects other than kinase inhibition may be important for TdP suppression with H-8. Alternately, inhibition of PKA (and perhaps other kinases) with H-8 shortens the QT interval. The suppression of TdP without shortening of the QT interval suggests that CaM kinase inhibitory agents may allow separation of the beneficial actions of QT prolongation (ie, increased inotropy and the class III antiarrhythmic effect) from the proarrhythmic actions (ie, afterdepolarizations and TdP). Although other mechanisms might be operative, the data presented here raise the strong possibility that direct targeting of intracellular kinases is feasible and produces important antiarrhythmic actions.

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References
20. Tanaka T, Ohnara T, Yamakado T, Hidaka H. Two types of calcium-dependent protein phosphorylations modulated by calmodulin antago-


33. Kadokami T, Shimokawa H, Fukumoto Y, Ito A, Takayanagi T, Egashira K, Takeshita A. Coronary artery spasm does not depend on the intracellular calcium store but is substantially mediated by the protein kinase C-mediated pathway in a swine model with interleukin-1\(


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