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Inhibition of Cardiac $\text{Ca}^{2+}$ Release Channels (RyR2) Determines Efficacy of Class I Antiarrhythmic Drugs in Catecholaminergic Polymorphic Ventricular Tachycardia

Hyun Seok Hwang, PhD; Can Hasdemir, MD; Derek Laver, PhD; Divya Mehra, BPharm; Kutsal Turhan, MD; Michela Faggioni, MD; Huiyong Yin, PhD; Björn C. Knollmann, MD, PhD

Background—Catecholaminergic polymorphic ventricular tachycardia (CPVT) is caused by mutations in the cardiac ryanodine receptor (RyR2) or calsequestrin (Casq2) and can be difficult to treat. The class Ic antiarrhythmic drug flecainide blocks RyR2 channels and prevents CPVT in mice and humans. It is not known whether other class I antiarrhythmic drugs also block RyR2 channels and to what extent RyR2 channel inhibition contributes to antiarrhythmic efficacy in CPVT.

Methods and Results—We first measured the effect of all class I antiarrhythmic drugs marketed in the United States (quinidine, procainamide, disopyramide, lidocaine, mexiletine, flecainide, and propafenone) on single RyR2 channels incorporated into lipid bilayers. Only flecainide and propafenone inhibited RyR2 channels, with the S-enantiomer of propafenone having a significantly lower potency than R-propafenone or flecainide. In Casq2$^{-/-}$ myocytes, the propafenone enantiomers and flecainide significantly reduced arrhythmogenic $\text{Ca}^{2+}$ waves at clinically relevant concentrations, whereas Na$^+$ channel inhibitors without RyR2 blocking properties did not. In Casq2$^{-/-}$ mice, 5 mg/kg R-propafenone or 20 mg/kg S-propafenone prevented exercise-induced CPVT, whereas procainamide (20 mg/kg) or lidocaine (20 mg/kg) were ineffective (n=5 to 9 mice, P<0.05). QRS duration was not significantly different, indicating a similar degree of Na$^+$ channel inhibition. Clinically, propafenone (900 mg/d) prevented CPVT in a 22-year-old CPVT patient who had been refractory to maximal standard drug therapy and bilateral stellate ganglionectomy.

Conclusions—RyR2 cardiac $\text{Ca}^{2+}$ release channel inhibition appears to determine efficacy of class I drugs for the prevention of CPVT in Casq2$^{-/-}$ mice. Propafenone may be an alternative to flecainide for CPVT patients symptomatic on $\beta$-blockers. (Circ Arrhythm Electrophysiol. 2011;4:128-135.)

Key Words: class I antiarrhythmic drugs • propafenone • RyR2
• catecholaminergic polymorphic ventricular tachycardia • flecainide • ranolazine • tetrodotoxin • quinidine • procainamide • disopyramide • lidocaine • mexiletine

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited arrhythmia syndrome characterized by physical or emotional stress-induced bidirectional or polymorphic ventricular tachycardia.

The more common autosomal-dominant form has been linked to mutations in the gene encoding the cardiac ryanodine receptor (RyR2) or calsequestrin (Casq2) and can be difficult to treat. The class Ic antiarrhythmic drug flecainide blocks RyR2 channels and prevents CPVT in mice and humans. It is not known whether other class I antiarrhythmic drugs also block RyR2 channels and to what extent RyR2 channel inhibition contributes to antiarrhythmic efficacy in CPVT.

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Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited arrhythmia syndrome characterized by physical or emotional stress-induced bidirectional or polymorphic ventricular tachycardia. The more common autosomal-dominant form has been linked to mutations in the gene encoding the cardiac ryanodine receptor (RyR2) or calsequestrin (Casq2). A less common but more severe autosomal-recessive form is caused by mutations in the gene encoding cardiac calsequestrin (CASQ2). The major Ca$^{2+}$-binding protein in the sarcoplasmic reticulum (SR). Ventricular myocytes isolated from mouse models of both forms of CPVT exhibit catecholamine-induced premature SR Ca$^{2+}$ release and spontaneous Ca$^{2+}$ waves that trigger delayed afterdepolarizations (DADs) and premature beats. Thus, the spontaneous opening of SR Ca$^{2+}$ release channels elicited by catecholaminergic stress are the likely culprit for triggering ventricular arrhythmias in CPVT.

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Although significantly reduced, cardiac events remain unacceptably high in CPVT patients treated with $\beta$-blockers. Even implantable cardioverter-defibrillators (ICDs) are not necessarily effective, because defibrillation shocks can cause catecholamine release and electrical storm, and deaths have been reported in CPVT patients with ICDs. Thus, there is a need for better drug therapy in CPVT. We recently found that the class Ic antiarrhythmic drug flecainide directly targets the molecular defect in CPVT by open-state block of RyR2 channels and prevents CPVT in mice and humans.
ever, it is not known whether other class I antiarrhythmic drugs also block RyR2 channels and to what extent RyR2 channel inhibition contributes to antiarrhythmic efficacy in CPVT. Thus, we tested the effect of all class I antiarrhythmic drugs currently marketed in the United States on single RyR2 channels incorporated into lipid bilayers. We found that only propafenone and flecainide inhibit RyR2 channels. The potency of RyR2 block determined antiarrhythmic activity of Na⁺ channels blockers in vitro and in vivo in Casq2−/− mice, a mouse model of CPVT. Clinically, propafenone prevented exercise-induced VT and recurrent ICD shocks in a highly-symptomatic CPVT patient refractory to standard therapy. Thus, RyR2 channel inhibition appears to be important for antiarrhythmic drug efficacy in CPVT. Propafenone may be a promising therapeutic alternative to flecainide for CPVT patients.

Methods

Animal Model and Experimental Procedures

All experiments were approved by the institutional animal care and use committees at Animal Care and Use Committees of Vanderbilt University in the United States and University of Newcastle in Australia and performed in accordance with National Institutes of Health guidelines. Adult Casq2−/− mice (12 to 16 weeks old) were used for all experiments. Casq2−/− mice consistently develop ventricular tachycardia during exercise or after catecholamine challenge.6,14 All data were analyzed in blinded fashion regarding the treatment groups. Quinidine hydrochloride monohydrate, procainamide hydrochloride, disopyramide phosphate salt, lidocaine hydrochloride, mexiletine hydrochloride, flecainide aceturate, propafenone hydrochloride, ranolazine dihydrochloride, and tetradoxotin were obtained from Sigma (St Louis, MO). (+)-R-flecainide and (-)-R-propafenone were separated on a ChiralPak AD column (25×0.46 cm, Chiral Technologies, Exton, PA) using hexane and 2-propanol containing 0.4% diethylamine. The flow rate was 1 mL and UV absorption was monitored at 247 nm. The purity of these 2 enantiomers was >99%.13

Single RyR2 Channel Measurements

SR vesicles containing RyR2 channels were obtained from sheep hearts and were reconstituted into artificial lipid bilayers.16 During SR-vesicle incorporation, the cis (cytoplasmic) bath contained (in mmol) 250 Cs⁺ (230 CsCl, Cs₂SO₄, 20 CsCl), 1.0 CaCl₂, and 500 mmammitol; the trans (luminal) solution contained 50 Cs⁺ (30 CsCl, 20 CsCl₂, 20 CsCl₂) and 1.0 CaCl₂. After detection of channels in the bilayer the [Cs⁺] of the trans solutions was increased to 250 mmol/L by means of aliquot addition of 4 mol/L CsCl, 1.0 M CsOH, or CsCl, 1.0 M CsOH. The cytoplasmic solution was exchanged to one containing 2 mmol/L ATP and 0.1 mmol/L free Ca²⁺ (1 mmol/L CaCl₂, 4.5 mmol/L BAPTA) via bath perfusion. The perfusion allowed exposure of a single channel to multiple drugs and concentrations that could be applied in any sequence. Solutions were pH-buffered with 10 mmol/L N-tris (Hydroxyethyl)methyl-2-aminoethanesulfonic acid (TES, ICN Biomedicals), and solutions were titrated to pH 7.4 using CsOH (optical grade, ICN Biomedicals) and were redox-buffered with 5 mmol/L glutathione (ICN Biomedicals).

Electric potentials are expressed using standard physiological convention (ie, cytoplasmic side relative to the luminal side at virtual ground). Single-channel recordings were obtained using bilayer potential difference of ±40 mV. The current signal was digitized at 10 kHz and low-pass filtered at 1 or 2 kHz with a gaussian digital filter. Open probability (Po) as well as open and closed durations were measured by the 50% threshold detection method (Channel2 software by P.W. Gage and M. Smith, Australian National University, Canberra).

Cell Isolations and Ca²⁺ Fluorescence Recordings

Ventricular myocytes were isolated by a modified collagenase/protease method as described.6 All the experiments were conducted in Tyrode solution containing (in mmol: CaCl₂ 2, NaCl 134, KCl 5.4, MgCl₂ 1, glucose 10, and HEPES 10, pH 7.4. Final concentration of Ca²⁺ was 2 mmol/L. After isolation, myocytes were loaded with Fura-2 acetoxyethyl ester (Fura-2AM).6 Briefly, myocytes were incubated with Fura-2AM (2 μmol/L) for 6 minutes at room temperature to load the indicator in the cytosol. Myocytes were washed twice for 10 minutes with Tyrode solution. A minimum of 30 minutes was allowed for deesterification before imaging the cells. Ca²⁺ fluorescence ratios (F₉₀/F₅₀) were recorded and normalized relative to the mean of vehicle group. The ratiometric fluorescent records were analyzed using commercially available data analysis software (IonWizard, IonOptix, Milton, MA). Spontaneous Ca²⁺ waves were measured using the following protocol: Fura-2AM-loaded myocytes were field stimulated at 1 Hz for 20 seconds until they reach a steady Ca²⁺ transient height. Then, stimulation was switched off and myocytes were monitored for 40 seconds for the occurrence of spontaneous Ca²⁺ waves, followed by application of caffeine (10 mmol/L) for 5 seconds using a rapid concentration clamp system. Amplitudes of caffeine-induced Ca²⁺ transients were used as estimates of SR Ca²⁺ content. Analysis was carried out as recently described by us.14 A spontaneous SR Ca²⁺ wave was defined as any spontaneous increase of 0.07 ratiometric units or more from the diastolic F₉₀.

ECG Recordings of Exercise-Induced and Isoproterenol-Induced Ventricular Tachycardia in Mice

Treadmill Exercise Test

Exercise testing in conscious Casq2−/− mice was carried out as previously described.6 Briefly, mice were initially anesthetized (pentobarbital, 70 mg/g), and an ECG transmitter (Data Sciences International, St Paul, MN) was implanted into the abdominal cavity with subcutaneous electrodes in lead II configuration. Animals were allowed to recover for at least 6 days after surgery before participating in the treadmill exercise studies. Study drug or vehicle (DMSO) was injected intraperitoneally 30 minutes before exercise. We previously established that a dose of 20 mg/kg flecainide results in a serum flecainide concentration of 2.5 μmol/L 1 hour after intraperitoneal injection, causes a 25% increase in QRS duration, and effectively suppressed exercise-induced CPVT in mice.14 Because measurement of the QRS duration provides a rapid, noninvasive and accurate biomarker of Na⁺ channel block18 and because the objective of our study was to compare the efficacy of antiarrhythmic drugs at a similar degree of Na⁺ channel block, study drugs were administered at doses that produce a 25% increase in QRS duration (online-only Data Supplement Figure 1). Mice were placed individually into a special chamber of the motorized rodent treadmill (Exer-6M, Columbus Instruments, Columbus, OH) and exercised until they exhibited signs of exhaustion.6 For each mouse, treadmill testing was performed 4 times at an interval of >72 hours in randomized sequence. High-quality ECGs were recorded and analyzed from 15 minutes before exercise until 12 hours after as previously reported.14

Isoproterenol Challenge

The β-adrenergic receptor agonist isoproterenol was used to induce ventricular arrhythmia in anesthetized Casq2−/− mice as previously described.6 Briefly, mice were anesthetized with isoflurane vapor titrated to maintain the lightest anesthesia possible. On average, 1.0% vol/vol isoflurane vapor was sufficient to maintain adequate anesthesia. Loss of toe-pinch reflex and respiration rate was used to monitor levels of anesthesia. Baseline ECG was recorded for 5 minutes, followed by an additional 10 minutes after administration of isoproterenol (3 mg/kg ip). R-propafenone (5 mg/kg) or vehicle (DMSO) was injected intraperitoneally 30 minutes before administration of isoproterenol. Isoproterenol challenge was performed 2 times in the same mouse at an interval of >72 hours, with half the animals receiving first placebo, then R-propafenone, and the other half receiving first R-propafenone, then placebo.

Human Studies

Propafenone is an approved antiarrhythmic drug and was administered as part of routine clinical care. The patient’s legal guardian provided
Statistically significant if the probability value was

These experiments were carried out using
class lipid bilayers. These experiments were carried out using

We first tested the effect of all class I antiarrhythmic drugs

Among class I antiarrhythmic drugs, only flecainide, propafenone, and its enantiomers (S- and R-propafenone) inhibit RyR2 activity. A, Relative change in channel open probability caused by study drug application at a concentration of 20 μmol/L. B, Records are representative examples of single channel activity of RyR2 in lipid bilayers. The baseline current during channel closures is labeled “C” (dashed lines) and channel openings correspond to upward current transitions. Control conditions were 1 mmol/L luminal Ca2+ (trans bath), 0.1 μmol/L cytoplasmic Ca2+ plus 2 mmol/L ATP (cis bath). Bilayer potential was 40 mV (relative to trans bath as ground). Relatively high drug concentrations (50 μmol/L) were used to better illustrate their effects on RyR2 channel gating. Drug addition introduced short (∼1 ms) closures to a substrate, labeled “S” (dotted lines), at ∼50% of the full channel conductance. The full durations of the long (∼1 second) closed periods that are present in control and drug records are not seen on this time scale. C, Concentration-response relationship of RyR2 channel inhibition by R- and S-propafenone and flecainide. RyR2 channel open probability (Po), mean open time (To), and mean closed time (Tc) are expressed relative to values in absence of drug. D, Comparison of IC50 values determined by least-squares fitting of Hill curves to the Po and Tc concentration response data (n=7 to 13 channels per group). *P<0.05.

Statistical Analysis

Differences between the groups of conscious mice were assessed using 1-way ANOVA with repeated-measure analysis conducted with SPSS, version 17.0 (SPSS Inc, Chicago, IL), followed by the Tukey post hoc test when appropriate. The incidence and rate of spontaneous Ca2+ waves in myocytes and ventricular ectopy in mice were compared using Fisher exact test and nonparametric Mann-Whitney test. Drug effects on single RyR2 channels were compared using 1-way ANOVA followed by the Tukey post hoc test when appropriate. The single-channel concentration response curves for S- and R-propafenone were compared by applying a t test to the differences between measured values and a common Hill curve over the concentration range of 10 to 50 μmol/L. Results were considered statistically significant if the probability value was <0.05.

Results

Propafenone Inhibits RyR2 Ca2+ Release Channels by Open State Block

We first tested the effect of all class I antiarrhythmic drugs currently marketed in the United States (quinidine, procainamide, disopyramide, lidocaine, mexiletine, flecainide, and propafenone) on single RyR2 channels incorporated into artificial lipid bilayers. These experiments were carried out using
cytoplasmic and luminal [Ca2+] that mimic cellular [Ca2+] during diastole (control condition: 2 mmol/L ATP, [Ca2+]cyt = 0.1 μmol/L, and [Ca2+]lum = 1 mmol/L). Although class Ia and Ib drugs had no significant effect on RyR2 channels, both class Ic drugs flecainide and propafenone significantly inhibited RyR2 channels at a concentration of 20 μmol/L (Figure 1A).

Propafenone is used clinically as a racemic mixture of S- and R-enantiomers. Both enantiomers are equipotent Na+-channel inhibitors. Only S-propafenone has moderate β-adrenergic receptor–blocking activity. Thus, we next examined the effects of R- and S-propafenone on RyR2 channels. A representative example of RyR2 channel opening under these control conditions is shown in Figure 1B (top trace). RyR2 open probability (Po) was ∼0.1. Addition of S-propafenone, R-propafenone, and flecainide to the cytoplasmic bath caused similar effects on RyR2 channel gating (Figure 1B). The reduction in RyR2 Po was primarily the consequence of reduced channel Tc, because all 3 drugs induced brief channel closures to a subconductance state with ∼30% of the open state conductance (Figure 1B and 1C). The concentration dependencies of R-propafenone, S-propafenone, and flecainide on Tc are shown in Figure 1C. Note that none of the 3 drugs significantly changed channel closed times (Tc, Figure 1C), which is in contrast to the RyR2 channel inhibitor tetracline that is not effective in CPVT myocytes. R-propafenone and flecainide reduced RyR2 Po with similar potency, whereas S-propafenone was significantly

Patient data collection was approved by the institutional ethics committee. ICD interrogations and analysis were performed every 2 months on a regular basis or immediately if the patient received ≥2 ICD shocks.

Patient consent for retrospective data analysis, left and subsequently right stellate ganglionectomy, and exercise stress testing. Patient data collection was approved by the institutional ethics committee. ICD interrogations and analysis were performed every 2 months on a regular basis or immediately if the patient received ≥2 ICD shocks.

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less potent (Figure 1D). Thus, RyR2 channel block by propafenone is stereoselective. The mechanism of propafenone action is consistent with an open state block and similar to flecainide.

**Only Na\(^+\) Channel Inhibitors With RyR2 Channel Blocking Properties Reduce Isoproterenol-Induced Ca\(^{2+}\) Waves in Casq2\(^{-/-}\) Myocytes**

We next tested the efficacy of class I antiarrhythmic drugs and other Na\(^+\) channel inhibitors in ventricular myocytes isolated from a mouse model of CPVT, Casq2\(^{-/-}\) mice. All study drugs were applied for 30 minutes before measurements were obtained. As previously reported,\(^6\) β-adrenergic stimulation with isoproterenol causes spontaneous Ca\(^{2+}\) waves (SCW) and triggered beats in Casq2\(^{-/-}\) myocytes (Figure 2A). Compared with treatment with vehicle, the rate of isoproterenol-stimulated Ca\(^{2+}\) waves was drastically reduced by application of either R-propafenone or flecainide (Figure 2A and 2B), with an IC\(_{50}\) of approximately 1.1±0.5 μmol/L and 2.0±0.2 μmol/L, respectively. Consistent with its lower potency in single RyR2 channels (Figure 1C), S-propafenone was significantly less effective compared to flecainide and R-propafenone in intact myocytes (S-propafenone, 6 μmol/L 0.41±0.12; P<0.05; Figure 2B). Class I agents that did not reduce RyR2 channel open probability (Figure 1A) had also no significant effect on isoproterenol-induced SCW in Casq2\(^{-/-}\) myocytes (Figure 2A and 2B). Moreover, the selective Na\(^+\) channel blocker tetrodotoxin and the late Na\(^+\) current inhibitor ranolazine also had no significant effect on SCW rate (Figure 2B). At the same time, none of the drugs tested significantly changed amplitude and decay rate of field-stimulated Ca\(^{2+}\) transients (data not shown) or SR Ca\(^{2+}\) content (Figure 2C). The latter is important because SR Ca\(^{2+}\) content is a determinant of the incidence of spontaneous Ca\(^{2+}\) waves.\(^{21}\) Taken together, these results demonstrated that RyR2 channel block is required to suppress arrhythmogenic Ca\(^{2+}\) waves, whereas Na\(^+\) channel block by itself has no significant effect.

**RyR2 Channel Block Is Required for Antiarrhythmic Efficacy of Class I Drugs in CPVT Mice**

To test the therapeutic efficacy of R-propafenone in vivo, we first studied anesthetized Casq2\(^{-/-}\) mice challenged with
isoproterenol (3 mg/kg). Casq2^{−/−} mice exhibit catecholamine-induced and exercise-induced polymorphic or bidirectional ventricular tachycardia. Analogous to flecainide, pretreatment with R-propafenone (5 mg/kg i.p.) prevented isoproterenol-induced ventricular ectopy (Figure 3). Ventricular extrasystoles occurred in 9 of 14 Casq2^{−/−} mice pretreated with vehicle (ventricular extrasystoles per minute, 4.6±1.6), but only in 1 of 14 Casq2^{−/−} mice pretreated with R-propafenone (ventricular extrasystoles per minute, 0.3±0.3, Mann-Whitney test, P=0.01). Interestingly, R-propafenone treatment significantly reduced the isoproterenol-induced heart rate response (peak heart rate [bpm]: Veh, 558±11 versus R-prop, 476±10; P<0.05).

Next, we examined to what extent RyR2 and Na\(^{+}\) channel block contribute to antiarrhythmic efficacy in vivo. To address this question, we compared the effect of 2 groups of drugs on exercised-induced CPVT of conscious Casq2^{−/−} mice: (1) class I antiarrhythmic drugs without RyR2-blocking properties (procaainamide and lidocaine, Figure 1A); and (2), class I drugs with RyR2-blocking properties (S- and R-propafenone). Both propafenone enantiomers are equipotent as Na\(^{+}\) channel blockers, but S-propafenone had lower potency on RyR2 channels (Figure 1C) and correspondingly lower efficacy in suppressing SCW in myocytes (Figure 2A). All drugs were dosed to produce a similar increase in QRS duration (online-only Data Supplement Figure 1), an in vivo marker of Na\(^{+}\) channel inhibition. Pretreatment with a single injection of R-propafenone (5 mg/kg) completely prevented VT during and for up to 4 hours after treadmill exercise, whereas procainamide (20 mg/kg) or lidocaine (20 mg/kg) had no significant effect on exercise-induced VT (Figure 4). The time course of R-propafenone reduction of VT is shown in online-only Data Supplement Figure 2. Consistent with its lower potency on RyR2 channels in vitro (Figure 1D), a 4-fold higher dose of S-propafenone (20 mg/kg) than R-propafenone was required to suppress VT in vivo (Figure 4). Collectively, these results indicated that RyR2 inhibition is required for antiarrhythmic efficacy of class I agents in CPVT mice. Furthermore, the stereoselective action of R-propafenone as RyR2 inhibitor translates into a higher potency of preventing exercise or catecholamine-induced polymorphic ventricular tachycardia in vivo.

**Propafenone Suppressed ICD Discharges and Exercise-Induced Ventricular Tachycardia in a CPVT Patient**

We next tried propafenone in a 22-year-old CPVT patient (RyR2 missense mutation p.L4105F) who was refractory to therapy. His medical history was remarkable for cerebral palsy caused by birth hypoxia. Within 24 hours of admission, the patient had polymorphic ventricular tachycardia associated with loss of consciousness requiring 4 direct-current cardioversions. Holter monitoring showed frequent polymorphic and bidirectional VT. The patient underwent placement of a single-chamber ICD in June 2007. Ventricular arrhythmias were initially controlled with the combination of metoprolol (200 mg/d) and verapamil (120 mg/d). Starting in the fall of 2008, the patient had frequent appropriate ICD discharges (91 shocks during the 6-month period from November 2008 until April 2009) despite maximal standard drug therapy and bilateral cardiac sympathetic denervation performed by stellate ganglionectomy (Figure 5). Because flecainide is not available for clinical use in Turkey, where the patient lives, propafenone (initially 300 mg/d, subsequently 600 mg/d, and finally 900 mg/d) was started in April 2009 and resulted in a dramatic reduction of ICD discharges (Figure 5). Only 2 episodes of ICD discharges occurred during 12 months of follow-up, after the propafenone dose was increased to 600 to 900 mg/d. Propafenone therapy also completely prevented ventricular arrhythmias during treadmill exercise testing (online-only Data Supplement Figure 3).
Discussion

Only Class Ic Antiarrhythmic Drugs Propafenone and Flecainide Directly Target Molecular Defects in CPVT Patients

Over the last decade, the disease mechanism responsible for the CPVT has been well established.8 Mutations in RyR2 or Casq2 cause a sensitization of the RyR2 Ca\(^{2+}\) release channel complex to SR luminal Ca\(^{2+}\). As a result, RyR2 channels open prematurely under conditions of high SR Ca\(^{2+}\) load, which typically occurs in conditions of \(\beta\)-adrenergic stimulation and/or fast heart rates.25 The premature Ca\(^{2+}\) release from dyadic RyR2 channel clusters triggers a propagated Ca\(^{2+}\) wave, which in turn activates the electrogenic NaCa exchanger located on the cell membrane.26 The ensuing NaCa exchange produces a net inward Na\(^{+}\) current, which depolarizes the cell membrane and generates a DAD.27 DADs of sufficient amplitude activate voltage-gated Na\(^{+}\) channels and trigger full action potentials. Given its unique pharmacological properties, propafenone has multiple modes of action in CPVT: The RyR2 open channel block discovered here will reduce the likelihood of RyR2 opening triggering an arrhythmic Ca\(^{2+}\) wave.13 The class Ic–type tonic Na\(^{+}\) channel inhibition during diastole will decrease the likelihood of any remaining DADs in triggering premature ventricular extrasystoles.28 Although less potent as a RyR2 inhibitor, the \(\beta\)-blocking activity of the S-enantiomere will also contribute to clinical efficacy of propafenone racemate in CPVT. These multiple modes of propafenone action may explain why propafenone was effective in a patient refractory to all currently known pharmacological and surgical therapy in CPVT.

RyR2 Open Channel Block by Class Ic Antiarrhythmic Agents

It is interesting to note that local anesthetics and other drugs that inhibit Na\(^{+}\) channels often also modulate RyR2 channels, albeit with a large variety of effects. In the present study, we show that flecainide and propafenone effectively inhibit RyR2 by reducing channel mean open durations via an open-state blocking mechanism. The RyR2 inhibitory action was not shared by any of the clinically available class Ia or Ib antiarrhythmic agents. Other anesthetics such as tetracaine, procaine, and QX314 also inhibit RyR2 channels29 but have a significantly lower potency and a very different mode of action compared with that of propafenone and flecainide.13 Tetracaine, unlike flecainide and propafenone, causes long closures by binding to channels in their closed state, leading to substantially increased closed-channel intervals.13 The prototypical class Ib agent lidocaine has no effects on RyR2 channels (Figure 1A) and activates RyR2 channels at higher concentrations.30 The tricyclic antidepressant amitriptyline, which is a potent Na\(^{+}\) channel inhibitor, activates RyR2 channels at clinically relevant concentrations.16 Amitriptyline binds to RyR2 in their open state and induces prolonged channel openings with \(\approx80\%\) of the normal channel conductance.16

The open-state RyR2 channel block probably contributes to the effectiveness of flecainide and propafenone in suppressing DADs and triggered arrhythmias in experimental models.28 RyR2 channel inhibition may also explain the negative inotropic effects of propafenone and flecainide, which exceed that expected from Na\(^{+}\) channel block alone31 and the significant risk of aggravation of heart failure observed clinically with propafenone and flecainide.32

Implications for Drug Therapy in CPVT

Currently, \(\beta\)-blockers are the first line of therapy in CPVT.8 They are the only class of drugs that has been shown to be effective in improving survival in CPVT.9 Because the incidence of cardiac events remains considerable even on \(\beta\)-blockers9,33 and ICDs are not always effective,10–12 there is a need for alternative therapies. Small case series suggest that Ca\(^{2+}\) channels blockers34 or stellate ganglionectomy35 may provide additional benefit. However, as illustrated by CPVT patients reported here and previously,14 neither therapy appears to be completely effective in CPVT. Class Ia and Ib antiarrhythmic agents are not effective as shown here and previously reported by others in CPVT mouse models36 and humans.33 These results are not surprising because lidocaine can activate RyR2 channels30 and would be predicted to
worsen the underlying molecular defect. We recently reported that flecainide, a class Ic agent, appeared effective in a mouse model CPVT and in 2 patients with drug-refractory CPVT, possibly because of its dual mode of action. Although a recent study failed to find a significant effect of flecainide during an in vivo screen of antiarrhythmic drugs in CPVT mice,36 this study was not designed nor statistically powered to examine flecainide action.37 Another group recently confirmed flecainide efficacy in Purkinje cells.38 Our results with propafenone further support the hypothesis that open-state block of RyR2 channels13 coupled with Na+ channel inhibition during diastole presents a promising therapeutic approach in CPVT. Our finding that S-propafenone was less effective than R-propafenone in reducing exercise-induced ventricular tachycardia in CPVT mice suggests that the RyR2 blocking action may be more important than Na+ channel blocking activity for antiarrhythmic drug efficacy in CPVT. Because the propafenone racemate used clinically has also β-blocking properties, it may be a promising therapeutic option for CPVT patients who require life-long drug therapy.

Study Limitations

Our data do not exclude the possibility that in addition to the RyR2 inhibition discovered in the present study, block of Na+ channels, and/or other ion channel also contribute to the antiarrhythmic efficacy of propafenone in CPVT mice. The exact contribution of RyR2 channel block to propafenone action will have to be addressed by comparison with a selective open channel RyR2 inhibitor, an agent that is currently not available. Although our in vitro studies suggest that propafenone is a mechanism-based drug therapy in CPVT, our clinical experience is limited to a single patient. Given the proarrhythmic effects of class Ic agents in patients with ischemic heart disease or heart failure,39 the diagnosis of CPVT should be clearly established and structural heart disease ruled out. As with any new therapeutic regimen, future clinical studies have to be done to define the risks and benefits of propafenone as treatment for CPVT.

Conclusion

In this study, we have shown that among clinically available class I antiarrhythmic drugs, only flecainide and propafenone inhibit RyR2 channels, suppress arrhythmogenic Ca2+ waves, and prevent CPVT in Casq2−/− mice. Thus, the potency of RyR2 channel inhibition rather than Na+ channel block appears to determine efficacy of class I agents for the prevention of CPVT. Clinically, propafenone was effective in suppressing appropriate ICD shocks in a CPVT patient refractory to standard therapy. Thus, propafenone and flecainide both are promising mechanism-based drug therapies for CPVT patients. However, the stereoselective action of propafenone discovered in the present study has to be considered for its clinical use in CPVT patients, given the stereoselective metabolism of propafenone racemate in humans.40,41

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Disclosures

None.

References

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Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a rare familial arrhythmia syndrome characterized by emotional or physical stress-induced polymorphic or bidirectional ventricular tachycardia. CPVT has been linked to mutations in genes that regulate Ca$^{2+}$ release from the sarcoplasmonic reticulum (eg, RYR2, CASQ2). Although β-blockers are first-line therapy, they are not always effective, and better drug therapy is needed. We recently discovered that the class I antiarrhythmic drug flecainide directly targets the molecular defect in CPVT by blocking RyR2 Ca$^{2+}$ release. RyR2 channel inhibition importantly contributes to antiarrhythmic efficacy in CPVT and should be considered when selecting drug therapy for CPVT patients. As illustrated by the CPVT case report, propafenone may be a promising alternative to flecainide for CPVT patients whenever flecainide is not clinically available or not tolerated. However, RyR2 channel inhibition by propafenone is stereoselective, with R-propafenone being significantly more potent than S-propafenone. Because propafenone is available clinically only as racemate and its metabolism is also stereoselective, alternative to flecainide for CPVT patients whenever flecainide is not clinically available or not tolerated. However, RyR2 channel inhibition by propafenone is stereoselective, with R-propafenone being significantly more potent than S-propafenone. Because propafenone is available clinically only as racemate and its metabolism is also stereoselective, large interindividual differences in clinical response may be expected when treating CPVT patients with propafenone.
Supplemental Figure 1. Effect of class I antiarrhythmic drugs on QRS duration in conscious CPVT mice. Procainamide (n=5, 20mg/kg), lidocaine (n=5, 20mg/kg), R-propafenone (n=9, 5mg/kg), or S-propafenone (n=5 per dose, 5mg/kg, 20mg/kg) were injected intraperitoneally 30 minutes before exercise. (A) Representative QRS complexes at baseline and 30 min after drug injection. (B) Average drug-induced increase in QRS duration. * vs. Vehicle p<0.05, ** vs. S-propafenone (5mg/kg) p<0.05.
Supplemental Figure 2. Time course of R-propafenone action of preventing exercise-induced ventricular tachycardia (VT) in conscious Casq2-/- mice. R-propafenone (5mg/kg), or vehicle (DMSO), were injected intraperitoneally (i.p.) 30 minutes before exercise. Data are mean and s.e.m., n=9 mice. One-way ANOVA with repeated measure analysis conducted with SPSS. * p<0.05 vs. Vehicle, “0” indicates No VT occurrences.
**Supplemental Figure 3.** Heart rate (HR) trend during exercise stress testing by Bruce protocol while the patient was on 200 mg/day of metoprolol and 600 mg/day of propafenone. Patient was able to walk 10 minutes and 26 seconds (12.9 METS). Peak heart rate was 88/beats per minute as shown in lead II rhythm strip. Note that combination therapy of propafenone and metoprolol completely prevented exercise-induced ventricular ectopy (VE).