Possible Targets of Therapy for Catecholaminergic Polymorphic Ventricular Tachycardia

– Insight From a Theoretical Model –

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Background: Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a serious disease with a high mortality but its management is limited. The aim of this study was to investigate specific target sites for therapy in order to find potential management strategies for CPVT.

Methods and Results: The mutant Ryanodine receptor 2 (RyR2) with reduced stored-overloaded-induced Ca^{2+} release (SOICR) threshold was incorporated into the Luo-Rudy dynamic (LRd) cell model to elucidate the underlying pathologies of CPVT. The simulations reveal that β -adrenergic stimulation increased the Ca^{2+} load in cardiac myocyte, which facilitates spontaneous SR Ca^{2+} leakage, resulting in triggered arrhythmias. Varied blockade (from 0% to 90%) in specific ion channels, including the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (I_{NaCa}), fast Na^+ channel (I_{Na}), RyR2 receptor (I_{rel}), Ca^{2+} -ATPase (SERCA) (I_{up}) or L-type Ca^{2+} channel ($I_{\text{Ca(L)}}$), was performed to simulate the action of specific drugs on target sites. Blockade of the I_{NaCa} ($\leq 10\%$ blockade), in contrast to the I_{up} ($\leq 30\%$ blockade), $I_{\text{Ca(L)}}$ and I_{Na} ($\leq 40\%$ blockade), and followed by I_{rel} ($\leq 80\%$ blockade), was most effective in suppressing the triggered arrhythmias in CPVT. Specifically, dual blockade of $I_{\text{Ca(L)}}$ / I_{up} , I_{Na} / I_{rel} or $I_{\text{Ca(L)}}$ / I_{rel} had a synergistic effect in CPVT management.

Conclusions: Blockade of I_{NaCa} appears to be the most efficacious target for CPVT management. Dual blockade of $I_{\text{Ca(L)}}$ / I_{up} , I_{Na} / I_{rel} or $I_{\text{Ca(L)}}$ / I_{rel} has a synergistic effect in CPVT treatment. (*Circ J* 2011; **75**: 1833–1842)

Key Words: Computational biology; Delayed afterdepolarization; Ventricular arrhythmias

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a familial arrhythmogenic disease with multifocal ventricular arrhythmias in the absence of structural heart disease under high β -adrenergic tone, which can sometimes deteriorate to ventricular fibrillation and sudden cardiac death.^{1,2} CPVT is a serious disease with a high mortality rate and its management is limited. The β -adrenergic blockers are the mainstay of the pharmacological management of CPVT. Unfortunately, a remarkable cardiac event recurrence rate with frequent ventricular arrhythmias (~30%) is still noted for patients with β -blocker compliance. Identification of adjunctive therapy able to reduce the burden of life-threatening arrhythmias in CPVT is therefore warranted.

At present, the precise mechanistic basis for both the arrhythmogenesis and treatment in CPVT remains debatable. We therefore used a detailed Luo-Rudy dynamic (LRd) cell model of a mammalian ventricular myocyte to investigate

the hypothesis that enhancing luminal Ca^{2+} sensing due to a reduced stored-overloaded-induced Ca^{2+} release (SOICR) threshold^{3–5} for the mutant Ryanodine receptor 2 (RyR2) promotes delayed afterdepolarizations (DADs) in CPVT. Specifically, the goals of this study were: (1) to evaluate the underlying electrophysiological mechanisms by which the “gain of function” of RyR2 mutations results in CPVT expression, (2) to investigate the role of reduced SOICR threshold in triggering arrhythmias at the whole cell level under β -adrenergic stimulation; and (3) to evaluate several possible targeted sites of anti-arrhythmic therapy in the CPVT mutant cell to find a potential management strategy.

Methods

The LRd cell model incorporating detailed L-type Ca^{2+} channel ($I_{\text{Ca(L)}}$) and RyR2 (I_{rel}) Markov chain models provides

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Table 1. Effect of Stepwise Reduction in the SOICR Threshold of Mutant RyR2 on RMP, APD₉₀, Peak Systolic [Ca²⁺]_i and DAD Number Between Paced AP (BCL=1,000 ms) in M-Cell

SOICR threshold (mmol/L)	RMP (mv)	APD90 (ms)	[Ca ²⁺] _i (10 ⁻³ mmol/L)	DAD (1/s)
No β-adrenergic stimulation				
7	-88.4	248	1.17	0
5	-88.4	248	1.17	0
3	-88.4	248	1.17	0
1	-88.9	250	0.74	1
With β-adrenergic stimulation				
7	-78.1	296	3.89	0
5	-80.39	309	2.22	1
3	-78.55	309	1.04–1.71	2
1	-78.26	310	0.59	>5

Midmyocardial (M-) cell.

SOICR, stored-overloaded-induced Ca²⁺ release; RyR2, Ryanodine receptor 2; RMP, resting membrane potential; APD, action potential duration; DAD, delayed afterdepolarization; AP, action potential; BCL, basic cycle length.

the basis for simulations since it contains detailed intracellular Ca²⁺ cycling, calcium-induced calcium release (CICR) process and SOICR events.^{6–10} The formulation of I_{rel} flux can be expressed as:⁶

$$I_{rel} = O * G_{rel} * ([Ca^{2+}]_{JSR} - [Ca^{2+}]_{ss})$$

$$G_{rel} = 250 * \left(\frac{graderel}{vgainorel} + soicr \right)$$

where O is the open probability of RyR2, G_{rel} is the conductance of the I_{rel} receptor, $[Ca^{2+}]_{JSR}$ and $[Ca^{2+}]_{ss}$ are the concentrations of free Ca²⁺ in the junctional SR (JSR) and subspace, respectively, $\frac{graderel}{vgainorel}$ and $soicr$ represent the gating variable of CICR and SOICR events, respectively. Furthermore, $soicr$ can be calculated as a function of time course (dt) and $[Ca^{2+}]_{JSR}$:

$$soicr_{ss} = \frac{25}{(1 + \exp(\frac{6.8 - [Ca^{2+}]_{JSR}}{0.001}))}$$

$$soicr_{\tau} = 125$$

$$soicr = soicr_{ss} - (soicr_{ss} - soicr) * \exp(\frac{-dt}{soicr_{\tau}})$$

where $soicr_{\tau}$ is the time constant and $soicr_{ss}$ is the steady-state value of $soicr$. The formulation is successful in generating SOICR events, and thus DAD formation, which is in agreement with experimental results for the amplitude and duration of DADs.⁴ In the original formulation of SOICR,⁶ the number 6.8 (mmol/L) is defined as the “SOICR threshold”, which means that spontaneous Ca²⁺ release will happen if the $[Ca^{2+}]_{JSR}$ reaches this critical level.⁶ Under special pathological conditions such as CPVT, reduction of the SOICR threshold caused by the mutant RyR2 will make the $[Ca^{2+}]_{JSR}$ reach the critical point frequently, which can potentially induce more SOICR events. In our simulation, the SOICR threshold of the wild type cell was set at 8 mmol/L. The electrophysiological behavior of the RyR2 mutant cell under different severities was simulated via stepwise, gradual reduction of the SOICR threshold. In addition, β-adrenergic agonist stimulation with isoproterenol (ISO)^{6,11} was performed to simulate the effect of β-adrenergic stimulation in the mutant RyR2 cell. The β-adrenergic signal cascade substrates several channels including the $I_{Ca(L)}$, sarcoplasmic reticulum (SR)

Ca²⁺-ATPase (I_{up}), I_{rel} , the Na⁺-K⁺ pump (I_{NaK}), the inward rectifier K⁺ current (I_{Kr}), and the slow delayed rectifier K⁺ current (I_{Ks}),^{6,11} which are formulated into the LRd cell to simulate the electrophysiological process of mutant RyR2 cell under β-adrenergic stimulation. As well, action potential (AP) simulations are performed by setting the density ratios between the slow and rapid delayed rectifier K⁺ currents (I_{Ks}/I_{Kr}) with 23:7:15 in the epicardial (epi-), midmyocardial (M-), and endocardial (endo-) cells to simulate the heterogeneity of the myocardium.¹²

Results

Effect of Reduced SOICR Threshold and β-Adrenergic Stimulation in Single CPVT Mutant Cell

Stepwise reduction of the SOICR threshold from 7 to 1 mmol/L in the LRd M-cell under a basic cycle length (BCL) of 1,000 ms resulted in minimal change of the resting membrane potential (RMP) and the AP duration of 90% repolarization (APD₉₀) (Table 1), which are compatible with results of a previous study showing that the morphology of the monophasic AP (MAP) was not obviously different in CPVT patients and control subjects.¹³ Figure 1 illustrates the morphologies and time courses of the AP and I_{rel} , Ca²⁺ transients in the intracellular space, and junctional and network SR ($[Ca^{2+}]_i$, $[Ca^{2+}]_{JSR}$, and $[Ca^{2+}]_{NSR}$) of the LRd cell at 7, 5, 3 and 1 mmol/L SOICR threshold, respectively. The Ca²⁺ effluxes through the I_{rel} are contributed from the CICR ($I_{rel,CICR}$) or SOICR ($I_{rel,SOICR}$) events. The $I_{rel,CICR}$ flux occurs at the initiation of phase 2 AP in response to the activation of $I_{Ca(L)}$, while the $I_{rel,SOICR}$ flux can occur at any time once the $[Ca^{2+}]_{JSR}$ reached the SOICR threshold. The $I_{rel,SOICR}$ flux then spreads into the bulk myoplasm and contributes to the intracellular Ca²⁺ transient ($[Ca^{2+}]_i$). In our simulation, neither $I_{Ca(L)}$ nor DADs generation was noted when the SOICR threshold was higher than 3 mmol/L, since the maximal $[Ca^{2+}]_{JSR}$ does not exceed the critical point without isoproterenol (ISO) application. $I_{rel,SOICR}$, extrasystolic Ca²⁺ transient, and DADs were noted between paced AP once the SOICR threshold was reduced to less than 2 mmol/L. Of note, the emergence of a diastolic Ca²⁺ transient was accompanied by a decreased amplitude of the following systolic Ca²⁺ transient (0.74*10⁻³ mmol/L) seen in the mutant M-cell at 1 mmol/L of the SOICR threshold. The result is compatible with a previous study that showed that the

Figure 1. Whole cell action potential (AP), Ca^{2+} transient in the intracellular space (Cai), junctional SR (JSR) (CaJSR), network SR (NSR) (CaNSR), and I_{rel} (Ca^{2+} release flux of the SR from RyR2) of mutant RyR2 M-cell at different levels of SOICR threshold under slow pacing with BCL=1,000ms. The β -adrenergic stimulation (ISO–: blue; ISO+: red) is performed to investigate its role for the arrhythmogenic behaviors in RyR2 mutation. Stars show the $I_{\text{rel,CICR}}$ flux. Arrows show the $I_{\text{rel,SOICR}}$ flux. BCL, basic cycle length; ISO, isoproterenol; SOICR, stored-overloaded-induced Ca^{2+} release.

occurrence of diastolic Ca^{2+} waves depresses the subsequent systolic Ca^{2+} waves in the cardiac myocyte.¹⁴

The summary of the effects of ISO on APD₉₀, RMP, and peak systolic $[\text{Ca}^{2+}]_i$ in the M-cell with BCL=1,000ms at a variety of SOICR thresholds is shown in Table 1. Figure 1 illustrates the morphologies and time courses of AP, $[\text{Ca}^{2+}]_i$, I_{rel} , $[\text{Ca}^{2+}]_{\text{JSR}}$, and $[\text{Ca}^{2+}]_{\text{NSR}}$ of the mutant M-cell at 7, 5, 3, and 1 mmol/L SOICR threshold, respectively, under ISO application (ISO+). ISO application results in RMP elevation and APD prolongation for the mutant M-cell when BCL=1,000ms, effects that are mediated by ISO-dependent suppressed I_{K1} current at negative potentials and a greatly increased $I_{\text{Ca(L)}}$ current during the AP plateau, respectively. Of note, there is a slight trend of progressive APD₉₀ prolongation as the SOICR threshold is progressively reduced from 7 to 1 mmol/L (APD₉₀: from 296 to 310 ms), which is caused by the preceding diastolic Ca^{2+} transient suppressing the following systolic Ca^{2+} transient during the time course of AP, resulting in a further enhanced $I_{\text{Ca(L)}}$ current (data not shown) due to a depressed Ca^{2+} -dependent inactivation process. It is notable that the $[\text{Ca}^{2+}]_{\text{NSR}}$ does not increase with gradually reducing SOICR threshold, reflecting that RyR2 mutations

do not result in a significant change in the SR Ca^{2+} load when compared with WT RyR2s.¹³ As shown in Figure 1, ISO application provokes DADs at a variety of reduced SOICR thresholds. These DAD episodes are caused by aberrant Ca^{2+} release, seen as spontaneous oscillations of the Ca^{2+} in the intracellular and JSR compartments, which is mediated by repetitive SOICR events (Figure 1, stars). The DAD event between paced AP appears clinically as the late-appearing U-wave in the surface ECG.¹³ The $I_{\text{rel,SOICR}}$ and DAD events occur once the SOICR threshold is reduced to less than 6 mmol/L (data not shown), since the maximal $[\text{Ca}^{2+}]_{\text{JSR}}$ has exceeded its threshold point under the influence of ISO stimulation. Note that the frequencies of SOICR and DAD events are greater with gradually reducing SOICR threshold. For example, there is only one SOICR and DAD event between paced AP at 5 mmol/L of SOICR threshold. However, the SOICR and DAD events increase up to 2–3 beats between paced AP when the SOICR threshold is reduced to 3 mmol/L. Of note, the DAD amplitude is so small at the 1 mmol/L SOICR threshold, resulting in fibrillation-like RMP between paced AP. The multiple DAD episodes between paced AP in our simulation is consistent with a previous study revealing that

Table 2. Effect of Stepwise Reduction in the SOICR Threshold of Mutant RyR2 on TDR, APD ₉₀ and DAD Number Between Paced AP in Endo-Cell, M-Cell, and Epi-Cell (BCL=1,000 ms)							
SOICR threshold (mmol/L)	Endo-cell		M-cell		Epi-cell		TDR (ms)
	APD ₉₀ (ms)	DAD	APD ₉₀ (ms)	DAD	APD ₉₀ (ms)	DAD	
No β -adrenergic stimulation							
7	173	0	248	0	152	0	96
5	173	0	248	0	152	0	96
3	173	0	248	0	152	0	96
1	176	1	250	1	155	1	95
With β -adrenergic stimulation							
7	202	0	296	0	169	0	127
5	202	1	309	1	169	0	140
3	209	2	309	2	179	1–2	130
1	225	3	310	>5	191	3–4	119

Epicardial (Epi-), midmyocardial (M-), and endocardial (Endo-) cells.
TDR, transmural dispersion of repolarization. Other abbreviations see in Table 1.

some patients with CPVT exhibit occasional double and triple oscillations of the DADs between normal beats in the MAP recording.¹³

Table 2 summarizes the APD₉₀ and DAD frequency (number between paced AP) of the endo-, M- and epi-cells at a

variety of SOICR thresholds (BCL=1,000ms). With gradually reduction of the SOICR threshold, no obvious APD₉₀ prolongation is noted in all 3 cell types. The transmural dispersion of repolarization (TDR), defined as the interval between earliest (epi-cell) and latest (M-cell) repolarization

Figure 3. Intracellular ionic cycling and major ion channels underlying the induction of sustained DAD and DAD-mediated triggered activity (TA) for the mutant M-cell (SOICR threshold: 6 mmol/L) under ISO application. I_{rel} , Ca^{2+} release flux of the SR from RyR2; I_{NaCa} , Na^{+}/Ca^{2+} exchanger current; I_{Na} , fast Na^{+} channel current; I_{CaL} , L-type Ca^{2+} channel current; I_{Ks} , slow delayed rectifier K^{+} current. *Last paced beat at 50 s and a DAD is noted after stopping the pacing of the mutant cell. The solid arrow denotes the first TA. DAD, delayed afterdepolarization; ISO, isoproterenol; SOICR, stored-overloaded-induced Ca^{2+} release.

times of the 3 cell types, also shows no obvious prolongation as the SOICR threshold is reduced gradually. As shown in Table 2, β -adrenergic stimulation facilitates the APD_{90} prolongation in all 3 cell types. Under the influence of β -adrenergic stimulation, increasing DAD frequencies and a slight trend in APD_{90} prolongation are noted in all 3 cell types as the SOICR threshold is progressively reduced. Although TDR is enhanced under β -adrenergic stimulation, gradually reducing the SOICR threshold does not further prolong the TDR.

Abnormal Arrhythmogenesis of CPVT Mutant Cell

As summarized in Tables 1 and 2, with stepwise reduction in the SOICR threshold from 7 to 1 mmol/L, DADs are not noted in the mutant cell unless the SOICR threshold is reduced to 2 mmol/L without ISO application. With the application of ISO, DADs became inducible and their frequency increases when the SOICR threshold is progressively reduced. Of note, occasional beats of DAD-mediated triggered activity (TA) (data not shown) between paced AP are noted at the range of 6 to 3 mmol/L SOICR threshold. ISO application and rapid pacing facilitate DAD generation and sustained DAD-mediated TA at the reduced SOICR threshold. The emergence and morphology of sustained TA also depend on the degree

of reduction in the SOICR threshold. Figure 2 shows the post-pause response of a mutant M-cell after pacing for 50 s ($BCL=500$ ms) at different SOICR thresholds. In the simulation, non-sustained TA (lasting for 7 beats; SOICR threshold: 8 mmol/L), followed by sustained TA (SOICR threshold: 6 mmol/L) and then TA with trigeminy (SOICR threshold: 4 mmol/L) are noted. Of note, the threshold of DAD to develop TA does not vary with gradually reducing SOICR threshold. In fact, it is the amplitude of the DAD that determines if the TA can be triggered. Although the frequency and number of SOICR and DAD events increase with further reducing the SOICR threshold to 2 and 1 mmol/L, the amplitude of each DAD is not enough to reach the threshold of TA.

Figure 3 illustrates the corresponding intracellular ionic cycling and major ion channels during the whole time course of the sustained DAD-mediated TA for the mutant M-cell at the 6 mmol/L SOICR threshold, as shown in Figure 2. The occurrence of DAD is due to the spontaneous release of Ca^{2+} stores from the JSR upon exceeding its SOICR threshold, leading to $I_{rel,SOICR}$ efflux through the RyR2 then spread into the bulk myoplasm. The subsequent rise in $[Ca^{2+}]_i$ is removed by the forward mode I_{NaCa} , which generates a net

inward current and depolarizes the cell membrane to cause DAD formation. It is noted that the fast Na^+ channel (I_{Na}), $I_{Ca(L)}$ or I_{Ks} does not play a role in DAD generation. Persisting and perpetual DADs, accompanied by further depolarizing RMP caused by ISO stimulation, can be of sufficient magnitude to activate I_{Na} and $I_{Ca(L)}$, leading to the generation of TA. The Ca^{2+} storage in the SR is mainly balanced by the efflux of RyR2 (I_{rel}) and influx of SERCA (I_{up}). In general, there should not be sustained DADs or TA after stopping pacing the cardiac myocyte, since the repetitive release of I_{rel} flux in the quiescent cell will deplete the SR Ca^{2+} storage gradually and thus finally self-terminate the process. However, if several beats of TA are provoked by the DADs, the activation of $I_{Ca(L)}$ can provide the myocyte with additional Ca^{2+} load, which will be partially taken up into the SR mediated by I_{up} . If the influx of I_{up} (due to increased intracellular Ca^{2+} load) and the efflux of I_{rel} (due to reduced SOICR threshold) are in balance, the Ca^{2+} storage in the SR ($[Ca^{2+}]_{NSR}$) will reach a new steady point, ensuring maintenance of sustained TA without external electrical stimulation. This is a self-perpetuating process since the initial TA will induce more TA, a process we call “triggered activity begets triggered activity”.

Possible Target Sites for Anti-Arrhythmic Intervention

Several target sites for anti-arrhythmic therapy, including the I_{Na} , $I_{Ca(L)}$, I_{up} , I_{rel} , or I_{NaCa} , are focused on here to see if blockade of these channels can suppress the DAD-mediated arrhythmias of the mutant RyR2 cell. The simulation is performed in the mutant M-cell at 6 mmol/L SOCIR threshold under BCL=500ms and ISO application. Sustained TA is noted after pacing the mutant cell for 50s. The frequency of sustained DADs and DAD-mediated TA (1/min) were calculated based on the 60-s pause following the previous train of pacing, which is illustrated in Figure 4. A 10% stepwise blockade of the above channels from 0% to 90% was performed to see if the frequency of post-pause DADs and TA could be attenuated by the above interventions. As noted in Figure 5, only 10% blockade of I_{NaCa} was enough to totally eliminate the TA (from 96 to 0 beats/min) and obviously attenuate the frequency of DADs+TA (from 126 to 16 beats/min). The 30% blockade of I_{up} or 40% blockade of $I_{Ca(L)}$ also contributed to nearly total elimination of TA (from 96 to 0–2 beats/min), followed by 40% blockade of I_{Na} resulting in suppression of TA with residual captured beats (from 96 to 10 beats/min). By contrast, it needed up to 80% blockade of I_{rel} to obviously suppress the frequency of TA (from 96 to 5 beats/min).

Figure 5. Effects of possible target sites to suppress the frequency (1/min) of ISO-induced sustained DADs and DAD-mediated triggered activity (TA) in the mutant RyR2 M-cell at 6mmol/L of SOICR threshold, as shown in Figure 3. 10% stepwise blockade of individually targeted channels, $I_{Ca(L)}$, I_{Na} , I_{rel} , I_{NaCa} , and I_{up} from 0% to 90% blockade to evaluate the suppression of sustained DADs and TA (panel “DADs+TA” and “TA” (1/min)) under ISO application. DAD, delayed afterdepolarization; ISO, isoproterenol; SOICR, stored-overloaded-induced Ca^{2+} release.

Any 2 types of channel blockade in the same degree (10% stepwise blockade from 0% to 90%) were selected from I_{Na} , $I_{Ca(L)}$, I_{up} or I_{rel} to evaluate the potential efficacy of “combined” therapy in CPVT management. The I_{NaCa} was not selected since blockade of I_{NaCa} alone is strong enough (only ~10%) to inhibit arrhythmogenesis in the mutant M-cell. As noted in Figure 6, 20% dual blockade of $I_{Ca(L)}$ and I_{up} , in contrast to 40% single blockade of $I_{Ca(L)}$ or I_{up} , can suppress the frequency of DADs and TA efficiently (from 126 to 0 beats/min). Also, dual blockade of I_{Na}/I_{rel} (in the range of 0–90% blockade) or $I_{Ca(L)}/I_{rel}$ (in the range of 0–40% blockade) shows a propensity for a synergistic effect in suppressing the frequency of DADs and TA as compared to blockade of a single specific channel, respectively. By contrast, there is not any obvious synergistic effect seen in the dual blockade of I_{rel}/I_{up} , $I_{Na}/I_{Ca(L)}$, or I_{Na}/I_{up} .

Discussion

In this study we performed simulations to elucidate the possible mechanism underlying CVPT and thus potential management of the reduction of the SOICR threshold caused by RyR2 mutation. In summary, the important findings are: (1) a reduced SOICR threshold increases the propensity for SOICR and DAD events in mutant RyR2 cell, even without ISO (β -adrenergic) stimulation, (2) β -adrenergic stimulation leads to increased intracellular and SR Ca^{2+} load, which can further facilitate spontaneous SR Ca^{2+} release, resulting in DAD

generation and even TA. With a gradually reducing SOICR threshold, the frequency of SOICR events, intracellular Ca^{2+} oscillation, and DAD episodes between paced AP increases in all 3 cell types; (3) under the influence of β -adrenergic stimulation, progressive reduction in the SOICR threshold does not further enhance TDR, indicating that the transmural reentry seen in other ionic channelopathies, such as Brugada syndrome, congenital short or long QT syndrome, may not be relevant to the arrhythmogenesis in CPVT. Instead, the multiple and spreading DAD-mediated ectopic activities seen in the 3 types of cells at reduced SOICR thresholds play a major role in CPVT-related arrhythmias; and (4) targeting I_{NaCa} may be potentially useful as an adjunctive therapy to β -adrenergic blockers in suppressing CPVT-related arrhythmias. Specifically, dual blockade of $I_{Ca(L)}/I_{up}$, I_{Na}/I_{rel} or $I_{Ca(L)}/I_{rel}$ has a synergistic effect.

There are currently 3 possible biophysical/molecular mechanisms by which the RyR2 mutation increases its propensity for spontaneous Ca^{2+} release in CPVT. The first is the theory of decreased SOICR threshold due to the increased SR luminal Ca^{2+} sensitivity of the mutant RyR2,^{4,5,15,16} which is the mechanism we choose to model in our study. Other possible mechanisms include defective domain–domain interactions of RyR2¹⁷ and disruption of FKBP12.6 binding affinity.^{18,19} For the first hypothesis, it has been shown that defective domain–domain interactions (unzipping) between the N-terminal and central domains will destabilize the RyR2, facilitating conformational changes caused by binding of Ca^{2+} to the SR luminal

Figure 6. Effects of possible combined therapy to suppress the frequency (1/min) of ISO-induced uncaptured DADs and DAD-mediated triggered activity (TA) (DADs+TA) in the mutant RyR2 M-cell at 6mmol/L of SOICR threshold, as shown in Figure 3. 10% stepwise dual blockade of channels, $I_{Ca(L)}/I_{up}$, I_{Na}/I_{rel} , $I_{Ca(L)}/I_{rel}$, I_{rel}/I_{up} , $I_{Na}/I_{Ca(L)}$ and I_{Na}/I_{up} from 0% to 90% are applied to evaluate the suppression of sustained DADs and TA (frequency of DADs+TA (1/min)) under ISO application. DAD, delayed afterdepolarization; ISO, isoproterenol; SOICR, stored-overloaded-induced Ca^{2+} release.

Ca^{2+} sensor, and enhancing luminal Ca^{2+} activation,¹⁷ which is equivalent to the reduced SOICR threshold in our simulation. The second hypothesis proposes that RyR2 mutations reduce the binding affinity of RyR2 for the regulatory protein FKBP12.6, which destabilizes the closed state of RyR2 and thus predisposes to abnormal Ca^{2+} leakage during the diastolic phase of AP. Furthermore, the defective behavior can be exacerbated when protein kinase A phosphorylates RyR2 in response to β -adrenergic stimulation, which causes further Ca^{2+} leakage from the SR.¹⁹ The second hypothesis requires further experimental studies to describe how FKBP12.6 interacts with the RyR2, and how the interaction is interrupted by RyR2 mutation, before the development of a more detailed computational model. In addition, the second hypothesis is still currently conflicting because several recent studies showed that FKBP12.6-RyR2 interaction was unaffected in CPVT RyR2 mutants and that the arrhythmogenic behaviors of mutant RyR2 cell could not be suppressed after restoring the FKBP12.6 binding affinity for RyR2.^{3,15,16,20} Those results indicate that impaired FKBP12.6–RyR2 interaction may be mutation-specific or not even relevant to CPVT pathogenesis.

The therapy of CPVT remains challenging. In our study, we investigated potential therapy for CPVT in addition to β -adrenergic blockade. For the purpose of suppressing DADs and sustained TA in the mutant RyR2 cell, blockade of I_{NaCa} is most effective, followed by blockade of I_{up} , $I_{Ca(L)}$, or I_{Na} , and then blockade of I_{rel} . Some clinical studies indicate that

$I_{Ca(L)}$ channel blockers (ie, intravenous verapamil) have a beneficial adjunctive effect to β -adrenergic blockers by decreasing but not completely preventing the arrhythmic burden in CPVT.^{21–23} However, those studies only included small numbers of patients. Besides, relatively larger intravenous dosages would have to be prescribed to achieve the effect in clinical CPVT management, which may potentially compromise cardiac systolic function. In our simulation, a moderate degree of $I_{Ca(L)}$ blockade (~40%) was effective in suppressing CPVT-related tachyarrhythmias, which is primarily mediated by eliminating SOICR events via suppressing the SR Ca^{2+} load below the threshold. Furthermore, the combination of $I_{Ca(L)}$ and I_{up} blockade have an augmented effect in CPVT management due to their synergistic effect in reducing the SR Ca^{2+} load. Also, dual blockade of $I_{Ca(L)}$ and I_{rel} in the range of 0–40% showed a propensity for synergistic suppression in CPVT management. Combination therapy consisting of $I_{Ca(L)}/I_{up}$ or $I_{Ca(L)}/I_{rel}$ blocker may be tried in the future. Of note, our simulation revealed that the combination of I_{Na} and $I_{Ca(L)}$ blockade cannot further enhance their abilities in suppressing arrhythmogenesis in mutant RyR2 cell, suggesting that these channel blockers should not be used simultaneously for CPVT management in clinical practice (Figure 6).

Several recent experimental data obtained in a CPVT mouse model indicate that JTV519, an experimental drug that stabilizes the RyR2, does not show a significant anti-arrhythmic effect in CPVT management.²⁰ Although it may not be appro-

appropriate to see a RyR2 stabilizer as simply just a RyR2 “blocker”, as in our case, previous experimental results and our simulation indicate that the “single” target of the I_{rel} receptor via either stabilization or blockade may not be very efficacious in suppressing CPVT-related arrhythmias. By contrast, our simulation suggests that I_{rel} blocker may be a useful “adjuvant” when combined with a I_{Na} or $I_{Ca(L)}$ blocker (Figure 6). Otherwise, several studies reveal that the class IC antiarrhythmic drug, flecainide, is effective in CPVT management.^{21,24,25} The efficacy of flecainide is not only due to its blockade of I_{Na} (suppress TA) but also its ability to block I_{rel} (suppress DADs), which is compatible with our simulation showing the synergistic effect of dual blockade of I_{Na} and I_{rel} . This is important for flecainide dual channel blockade capability since other class I drugs with sole blockade of I_{Na} may be pro-arrhythmic and even harmful for ventricular arrhythmia management. Our simulation reveals that the management of CPVT should focus on drugs with potentially “multiple” target sites, if clinically available in the future.

In our simulation, blockade of I_{NaCa} was promising due to its ability to efficiently suppress the proarrhythmic DAD amplitude and thus further TA. However, it should be noted that there are several potential drawbacks with further high-level blockade of I_{NaCa} , including the increased propensity of Ca^{2+} mediated arrhythmogenesis (due to increased $[Ca^{2+}]_{NSR}$ load) and compromised diastolic function of the cardiac myocyte (due to increased resting $[Ca^{2+}]_i$). The I_{NaCa} exchanger is electrogenic and can operate in the forward or reverse mode, which is dependent on $[Ca^{2+}]_i$, $[Na^+]_i$ and the membrane potential. In general, the I_{NaCa} exchanger predominantly functions in the forward mode, serving as the major Ca^{2+} transporter out of the myocyte and thus playing an essential role in relaxation along with I_{up} . Increased forward mode I_{NaCa} activity is noted in several conditions of Ca^{2+} overload to extrude excessive intracellular Ca^{2+} storage, which can cause membrane depolarization, diastolic DADs and triggered arrhythmias. Otherwise, during myocardial ischemia or reperfusion injury, the high intracellular Na^+ load reduces the driving force for Ca^{2+} efflux by forward exchange and facilitates the activity of reverse mode I_{NaCa} to mediate Ca^{2+} influx, which may result in Ca^{2+} overload and rapid cell death. In our simulation, blockade of I_{NaCa} should be seen as “equal” to blockade of dual mode I_{NaCa} , since we just multiplied by a factor of 10–100% before the I_{NaCa} current to simulate channel blockade by drug intervention. Although there is the fear that I_{NaCa} blockade may adversely affect intracellular Ca^{2+} handling as mentioned in previous papers,^{26,27} our simulation indicates that at least low-level I_{NaCa} blockade (ie, only ~10% blockade) may be adequate, safe and effective for CPVT management. There are currently no data available to speculate on the efficacy of I_{NaCa} blocker in CPVT management. Our favorable result of dual mode I_{NaCa} blockade indicates that it may play an important role in CPVT treatment in the future.

The reduction in the SOICR threshold in our model not only applies to the case of CPVT but also to several other cardiac pathologies such as myocardial hypertrophy, ischemia, reperfusion or failure.^{28,29} The reduced SOICR threshold due to acquired RyR2 dysfunction, accompanied by overexpression of the I_{NaCa} exchanger and further depression of the transient outward K^+ (I_{to}) and I_{K1} channels, which are commonly seen in the failing heart, may contribute to the enhanced propensity for DAD-mediated ventricular arrhythmias in these pathologies.³⁰ The strategy of CPVT treatment suggested by our simulation may provide new insight for the management of those diseases.

Study Limitations

First, the simulation is performed on the ventricular myocyte of a guinea pig. We choose the LRd cell model due to its established utility in elucidating the mechanisms of cardiac arrhythmia.^{6–10,31} However, it may not represent the real electrophysiological behaviors in the human ventricular myocyte. Second, the LRd cell model uses whole cell “common pool” model of CICR in which uniform SR Ca^{2+} release in the entire cell is assumed. The cell model cannot be used to simulate local molecular processes of CICR such as Ca^{2+} spark formation or single RyR2 stochastic property. Third, there is recent evidence showing that Purkinje cells, in contrast to ventricular myocytes may be the cellular source of arrhythmogenesis in CPVT.^{32–34} In our study, we cannot directly compare the arrhythmogenesis of Purkinje vs. ventricular cells due to there being no available computational model of the Purkinje cell for the guinea pig yet. However, several studies have revealed that Purkinje fibers resembles the APD rate relationships of M cells, which is compatible with our simulations showing that M-cell (and may also Purkinje cell) displays more propensity for arrhythmogenesis than the other cell types. A more comprehensive Purkinje cell model incorporated with β -adrenergic stimulation is needed to investigate the arrhythmogenic activity in CPVT more precisely.

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