Dantrolene Improves Survival After Ventricular Fibrillation by Mitigating Impaired Calcium Handling in Animal Models

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- *Background*—Resistant ventricular fibrillation, refibrillation. and diminished myocardial contractility are important factors leading to poor survival after cardiac arrest. We hypothesized that dantrolene improves survival after ventricular fibrillation (VF) by rectifying the calcium dysregulation caused by VF.
- *Methods and Results*—VF was induced in 26 Yorkshire pigs for 4 minutes. Cardiopulmonary resuscitation was then commenced for 3 minutes, and dantrolene or isotonic saline was infused at the onset of cardiopulmonary resuscitation. Animals were defibrillated and observed for 30 minutes. To study the effect of VF on calcium handling and its modulation by dantrolene, hearts from 14 New Zealand rabbits were Langendorff-perfused. The inducibility of VF after dantrolene administration was documented. Optical mapping was performed to evaluate diastolic spontaneous calcium elevations as a measure of cytosolic calcium leak. The sustained return of spontaneous circulation (systolic blood pressure $\geq 60 \text{ mm Hg}$) was achieved in 85% of the dantrolene group in comparison with 39% of controls (P=0.02). return of spontaneous circulation was achieved earlier in dantrolene-treated pigs after successful defibrillation (21 ± 6 s versus 181 ± 57 s in controls, P=0.005). The median number of refibrillation episodes was lower in the dantrolene group (0 versus 1, P=0.04). In isolated rabbit hearts, the successful induction of VF was achieved in 83% of attempts in controls versus 41% in dantrolene-treated hearts (P=0.007). VF caused diastolic calcium leaks in the form of spontaneous calcium elevations. Administration of 20 µmol/L dantrolene significantly decreased spontaneous calcium elevation amplitude versus controls. (0.024 ± 0.013 versus 0.12 ± 0.02 arbitrary unit [200-ms cycle length], P=0.001).
- *Conclusions*—Dantrolene infusion during cardiopulmonary resuscitation facilitates successful defibrillation, improves hemodynamics postdefibrillation, decreases refibrillation, and thus improves survival after cardiac arrest. The effects are mediated through normalizing VF-induced dysfunctional calcium cycling. (*Circulation.* 2014;129:875-885.)

Key Words: cardiopulmonary resuscitation ■ death, sudden, cardiac ■ ryanodine receptor calcium release channel ■ ventricular fibrillation

Ventricular fibrillation (VF) is a major cause of sudden cardiac arrest and is the primary determinant of mortality from cardiovascular diseases. Nearly 83 000 cases of sudden cardiac death reported annually in United States are due to VF.¹ Cardiopulmonary resuscitation (CPR) and defibrillation are the mainstays of immediate management; however, despite significant progress in CPR techniques in past decades, mortality from VF has remained unacceptably high,^{2,3} with 23.8% surviving to hospital admission and only 7.6% survival to hospital discharge.³ Shock-resistant VF,

decreased cardiac contractility postdefibrillation (myocardial stunning), and the recurrence of VF (ventricular refibrillation) are the main challenges faced during CPR and they increase the morbidity and mortality.^{3,4}

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Studies of ion channel blockers have shown little or no benefit in experimental and clinical VF.² However, VF is associated with significant impairment in cardiac calcium cycling

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and results in intracellular calcium ($[Ca]^{2+}_{i}$) overload.⁵⁻⁷ Dysfunction in $[Ca]^{2+}_{i}$ handling and calcium overload during VF has been proposed to cause myocardial stunning postdefibrillation^{4,6} and trigger refibrillation by activating compensatory mechanisms such as the Na⁺/Ca²⁺ exchanger and causing after-depolarizations.⁸ Failed defibrillation or refibrillation leads to more ischemia, worsening the calcium overload. The stabilization of cardiac calcium cycling may be able to mitigate this vicious cycle, enhance defibrillation, and thus improve survival after VF.⁷

Ryanodine receptor-2 (RyR2) is the major calcium release channel expressed in the sarcoplasmic reticulum and plays a crucial role in cardiac contractility.⁹ Recently, it has been proposed that increased calcium leak from RyR2 results in the progression of heart failure and triggers VF.¹⁰ The stabilization of RyR2 function has been shown to be protective against cardiac arrhythmias in animal models of catecholaminergic polymorphic ventricular tachycardia.¹¹ Stabilizing RyR2 function and restoring normal calcium cycling during VF might facilitate defibrillation, improve cardiac contractility, and improve survival in VF.

Dantrolene sodium, a stabilizer of skeletal muscle RyR1, has recently been shown to bind to RyR2, restore sarcoplasmic reticulum calcium reserve, improve cardiac function, and prevent arrhythmogenesis in various animal models of heart failure.^{11–13} We hypothesized that the administration of dantrolene during CPR improves survival in an in vivo swine model of sudden cardiac arrest due to VF by (1) enhancing defibrillation success and organizing VF, (2) improving hemodynamics after defibrillation, and (3) decreasing the incidence of refibrillation. We further hypothesized that these effects of dantrolene are mediated via its effects on stabilizing cardiac calcium cycling that results from VF-induced dysfunction of [Ca]²⁺ handling.

Methods

In Vivo Swine Model

Healthy 10- to 12-week-old Yorkshire pigs with weights ranging between 27 and 34 kg (n=26) were used. The protocol was approved by the Animal Care Committee of St. Michael's Hospital. A more detailed description of the experimental model and measurements are provided in the online-only Data Supplement Materials. After initial stabilization, VF was induced by burst pacing (10 V of 60-Hz current for 2 s) and left untreated for 4 minutes. Then, chest compression was started by using a pneumatic device (Lucas, Jolife AB, Lund, Sweden) at 100 compressions per minute and manual ventilation at 6 breaths per minute with 5 to 6 L/min of 100% O2 with an AMBU bag was performed. CPR was continued for 3 minutes with no interruption of chest compressions. At the onset of CPR, animals received either a bolus dose of dantrolene sodium (2 mg/kg) or isotonic saline. The dantrolene dose was determined based on previous studies on in vivo swine models of malignant hyperthermia¹⁴ and other animal models.¹⁵ After 7 minutes of VF, defibrillation was attempted at 150J. If defibrillation was unsuccessful, CPR was immediately restarted and defibrillation was again attempted at 200J after 2 minutes (with subsequent defibrillation attempts at 300J→360J→360J→360J→360J in case of the failure of the second or subsequent shocks). The energy level delivered is based on the front-panel-indicated energy values on the defibrillator. After successful defibrillation, animals were monitored for 30 minutes to assess the outcome and occurrence of refibrillations. Refibrillation was treated with the same energy level that defibrillated the initial VF. If animals could not be defibrillated during refibrillation, the energy level was increased in a stepwise fashion to up to 4 attempts at 360J.

Ex Vivo Rabbit Langendorff Model

New Zealand white rabbits with weight ranging from 2.4 to 4.5 kg were used (n=14). Details on the model and measurements are provided in the online-only Data Supplement Materials. VF was induced by burst pacing for 30 s at 60 Hz and 10 V, and hearts were continuously perfused during VF. At 1 minute of VF, a single dose of dantrolene (20 µmol/L) or isotonic saline was infused in the bubble trap. VF was monitored for 4 minutes and then defibrillated at 5J. Five more episodes of fibrillation-defibrillation were tried on each heart with 3 minutes of recovery time (in sinus rhythm) in between. VF duration for each VF episode was 4 minutes (or shorter in the case of self-termination) followed by defibrillation. Simultaneous optical mapping of calcium and voltage was performed during pacing to evaluate calcium amplitude alternans. Alternans was defined as the beat-to-beat difference of >10% in calcium wave amplitude. Alternans threshold was defined as the longest pacing cycle length (PCL) at which alternans emerged. Diastolic spontaneous [Ca]²⁺, elevation (SCaE) as a measure for diastolic calcium leak from RyR2 was evaluated in rabbit hearts. The protocol to measure SCaE amplitudes was adopted from Lee et al.16

Isolated Cardiomyocytes and Mathematical Modeling

A detailed description of the isolated cardiomyocyte study and mathematical modeling is provided in the online-only Data Supplement Materials. In brief, isolated cardiomyocytes were paced at 10 Hz to mimic the high activation during VF in the presence of 100 nmol/L of isoproterenol. The effect of dantrolene on ameliorating the pacing-induced rapid activation of cardiomyocytes was evaluated. In computer simulations, the role of dantrolene in suppressing the VF-induced automaticity and action potential duration (APD) formation in the Purkinje system and the whole heart was assessed.

Statistical Analysis

Time to return of spontaneous circulation (ROSC), time to successful defibrillation, and the onset of refibrillation were analyzed by using the log-rank test for survival analysis. SCaE amplitudes, alternans threshold, organization index of calcium and APD waves, regularity index of VF signals, and systolic and diastolic pressure at different time points and between groups were compared by using 2-way repeated-measures analysis of variance with pairwise analysis using Sidak correction for multiple comparisons. Comparison of ordinal variables (number of refibrillations, number of defibrillation attempts) and continuous variables in unpaired groups was performed with the use of the Wilcoxon Mann-Whitney test. The Wilcoxon signed rank test was used to compare variables in paired groups. Repeated-measures logistic regression was used to compare the incidence of consecutive induced and sustained VF episodes in the rabbit hearts. Categorical variables were compared with the use of the Fisher exact test. P≤0.05 was considered statistically significant. For repeated-measures analysis between groups, the P value for treatment and time interaction is provided unless otherwise stated. For pairwise comparisons, the adjusted P values for multiple comparisons are provided. All statistical analyses were performed with the use of Stata 11.1 (Stata Corp LP) and SPSS 17.

Results

Dantrolene Increased Survival After VF

VF was successfully induced in all 26 animals. Pulsatile normal sinus rhythm immediately postdefibrillation (initial survival) was achieved in 85% (11/13) in the dantrolene group and 54% (7/13) in controls (P=0.1). At the end of the protocol, sustained ROSC was achieved in 85% (11/13) in the dantrolene group in comparison with 39% (5/13) in the controls (P=0.02; Table).

	Control	Dantrolene	P Value
Successful defibrillation*	85% (11/13)	100% (13/13)	0.24
Time to defibrillation,* s	351±37 Median: 420	231±19 Median: 180	0.006
Time to ROSC,† s	181±57	21±6	0.005
Total time in VF‡			
Excluding refibrillation	591±37	471±19	0.005
Including refibrillation	642±37	481±19	0.0009
Median number of defibrillation attempts (for initial VF)	3	1	0.042
Shock-resistant VF (>2 defibrillation attempts)	54% (7/13)	8% (1/13)	0.015
Maximum energy level	252±24J	181±16J	0.02
Total energy level	699±186	333±119	0.04
Refibrillation incidence§	71% (5/7)	27% (3/11)	0.08
Number of refibrillation episodes	1.5±0.6	0.5±0.4	0.04
Time to onset of refibrillation, s	421±301	55±11	0.22
Sustained ROSC after refibrillation	40% (2/5)	100% (3/3)	0.35
Mean number of shocks to terminate each refibrillation episode	2.2±0.5	1±0	0.05
Metabolic parameters			
pH (before defibrillation)	7.45±0.02	7.4±0.07	0.2
Pao, (before defibrillation)	130±51	189±30	0.4
Paco, (before defibrillation)	36±3.2	36±2.7	0.67

Table. Summary of Hemodynamic and Refibrillation Parameters in Dantrolene and Control Groups

BP indicates blood pressure; CPR, cardiopulmonary resuscitation; ROSC, return of spontaneous circulation; sBP, systolic blood pressure; and VF, ventricular fibrillation. *Successful defibrillation was defined as defibrillation of the initial VF into asystole, pulsatile, or pulseless sinus rhythm. Time to successful defibrillation was calculated from the onset of CPR.

†Time to ROSC was calculated from immediately postdefibrillation until sBP of >60 mm Hg was achieved.

‡Total time in VF includes the duration of initial VF plus all refibrillations observed during the experiment.

\$Data only include initial survivors of VF with normal sinus rhythm with any detectable BP immediately postdefibrillation. Analysis of time to ROSC, time to defibrillation, and time to refibrillation was performed by using the log-rank (Mantel-Cox) test for survival analysis.

Dantrolene Organized VF Signals and Facilitated Defibrillation

VF signals recorded at time 0 (immediately after the induction of VF), before CPR (4 minutes of VF), and immediately before the first defibrillation attempt (7 minutes of VF) were compared between the dantrolene and control groups (Figure 1). VF was significantly more organized at 7 minutes of VF in the dantrolene group versus controls as measured by the Regularity Index (RI) of VF signals (0.69 versus 0.55, *P*=0.004). VF organization significantly deteriorated during 7 minutes of VF in controls in comparison with dantrolene-treated pigs. (Δ RI= -0.1±0.04 in dantrolene versus Δ RI= -0.24±0.02 in controls, *P*=0.006; Figure 1).

In all animals combined (survivors and nonsurvivors), the dominant frequency of VF at time 0 was 7.6 ± 0.19 and 7.5 ± 0.26 Hz in dantrolene and control groups, respectively (*P*=0.53). At 7 minutes of VF (3 minutes after the initiation of CPR), the dominant frequency increased to 8.7 ± 0.62 and 9.9 ± 0.59 Hz in dantrolene and control groups, respectively (*P*=0.19). In survivors, the dominant frequency after 7 minutes of VF was significantly (*P*=0.047) lower in dantrolene-treated pigs than in controls (9.2±0.62 Hz and 11.2±0.61 Hz, respectively).

Hemodynamic Outcomes After CPR

Although successful defibrillation was achieved significantly (P=0.006) earlier in dantrolene-treated pigs, the time to ROSC was also shorter in the dantrolene group. ROSC (systolic

blood pressure [BP] \geq 60 mmHg) was achieved after 21±6 s postdefibrillation in the dantrolene group versus 181±57 s in controls (Figure 2).

Systolic and diastolic BP during the postdefibrillation period in the dantrolene group were significantly higher than in controls (P=0.0014 and P=0.0017, respectively; Figure 2). By the end of the experiments, mean systolic BP and diastolic BP was 85 (95% confidence interval, 77–93) and 61 (95% confidence interval, 54–68) mm Hg in survivors in the dantrolene group and 66 (95% confidence interval, 53–79) and 46 (95% confidence interval, 37–55) mm Hg in controls, respectively.

To account for different total duration of VF in the 2 groups overall, we compared the hemodynamic outcomes in a subgroup of animals that were successfully defibrillated with the first defibrillation attempt. The total duration of VF in these cases was 7 minutes (n=8 in the dantrolene group and n=4 in controls). Time to achieving a systolic BP \geq 60 mm Hg was shorter in the dantrolene group (26±8 s versus 93±48 s in controls, *P*=0.06). Systolic and diastolic BPs were significantly higher in dantrolene-treated pigs than in their control peers during the 30 minutes postdefibrillation period (*P*=0.0016 and *P*=0.002 for the treatment effect).

Dantrolene Decreased Refibrillation and Improved Outcomes After Refibrillation

The median number of refibrillation episodes was significantly lower in the dantrolene group than in controls.



Figure 1. Comparison of the temporal organization of VF between groups (in vivo experiments). **1**, The regularity index (RI) was measured at baseline (T0), 4 minutes of VF (before CPR), and 7 minutes of VF (3 minutes after drug infusion, before defibrillation; sample surface ECG tracing recorded from a control and a dantrolene-treated pig at 4 minutes of VF [before CPR] and at 7 minutes of VF [before first defibrillation attempt]). Lower RI values indicate higher disorganization of VF signals. **2**, Dantrolene administration during CPR mitigated or reversed the time-dependent disorganization of VF, whereas, in the control group, VF organization consistently deteriorated during 7 minutes of VF (*P*=0.031). RI at 7 minutes of VF was 0.69 (95% CI, 0.6–0.78) and 0.55 (95% CI, 0.49–0.6) in the dantrolene and control group, respectively (*P*=0.004). There was no statistical difference between RI at T0 and 4 minutes between groups (0.79 [95% CI, 0.72–0.86] vs 0.79 [95% CI, 0.75–0.83] [*P*=0.99] at T0 and 0.73 [95% CI, 0.68–0.78] vs 0.71 [95% CI, 0.63–0.79] at 4 minutes in dantrolene and control group, respectively; *P*=0.96). **3**, Dot plots demonstrating the percentage of change in dominant frequency (**top**) and regularity index (**bottom**) of VF after 7 minutes of VF from baseline (T0); dominant frequency increased by 35% in the control group and by 15% in dantrolene group (*P*=0.006]). CI indicates confidence interval; CPR, cardiopulmonary resuscitation; and VF, ventricular fibrillation.

Refibrillation occurred in 2 scenarios: when postdefibrillation rhythm was normal sinus rhythm with pulsatile rhythm (initial survivors) and when the postdefibrillation rhythm was either pulseless electric activity or asystole. Because the first scenario is more clinically relevant, we analyzed refibrillation in more detail in the initial survivors of the VF (Table). All refibrillations in the dantrolene group were terminated with the first defibrillation attempt and with no impact on hemodynamic parameters and survival afterward. Refibrillations were triggered by premature ventricular contractions in 96% of refibrillation episodes (24/25). Only in 1 case was refibrillation preceded by sustained ventricular tachycardia.

Dantrolene Did Not Alter Refractoriness

There was no significant difference in the postdefibrillation effective refractory period between dantrolene and controls $(215\pm9 \text{ ms versus } 206\pm4 \text{ ms}, P=0.47)$. In the dantrolene group,

the QT interval at baseline and postdefibrillation was 432 ± 48 ms and 334 ± 83 ms, but, in controls, the corresponding values were 442 ± 50 ms and 325 ± 6 ms, respectively. The change in postdefibrillation QT intervals versus baseline was not significant between control and dantrolene groups (*P*=0.35).

Ex Vivo Rabbit Protocol

Dantrolene-Treated Hearts Were Resistant to VF and Refibrillations

After the initial induction of 2 VF episodes, dantrolene-treated hearts were more resistant to refibrillation episodes. In controls, 20 (83%) of 24 attempts resulted in VF (VF lasting ≥ 10 s) in comparison with 41% (14/34 attempts) in dantrolene-treated hearts (*P*=0.007, repeated measures logistic regression). In several instances, VF episodes self-terminated into sinus rhythm or transitioned to monomorphic ventricular tachycardia in dantrolene-treated hearts. Additionally, spontaneous refibrillation occurred



Figure 2. Hemodynamic parameters (sBP, dBP, heart rate) throughout the experiment in the dantrolene and control groups in survived pigs. **1**, The data presented during the postdefibrillation period only include survivors (ROSC at 30 minutes after the termination of VF). **Left**, The changes in systolic BP. **Right**, changes in diastolic BP. VF0 indicates the BP during first minutes of VF; VF4, BP during the 4th minute of VF; CPR0, BP during the first minute of CPR; CPR3, BP during the 3rd minute of CPR. sBP was specifically higher at 5 minutes postdefibrillation in those treated with dantrolene ($103\pm7.2 \text{ mm}$ Hg [95% CI, 87-120 mm Hg] vs $58\pm5.8 \text{ mm}$ Hg [95% CI, 44-73 mm Hg]; *P*=0.002). Both sBP and dBP were significantly higher during the postdefibrillation period in dantrolene group vs controls. ****P*=0.0014 (for treatment effect) vs control; ***P*=0.0017 (for treatment effect) vs control. **2**, Kaplan-Meier curves comparing time with ROSC (sBP≥60 mm Hg) between groups after defibrillation. ROSC was achieved significantly earlier in the dantrolene group during the postdefibrillation in survivors. The heart rate was lower in the dantrolene group but was not statistically different from controls (*P*=0.12). CI indicates confidence interval; CPR, cardiopulmonary resuscitation; dBP, diastolic blood pressure; defib, defibrillation; ROSC, return of spontaneous circulation; sBP, systolic blood pressure; and VF, ventricular fibrillation.

in 4 control hearts and in none of the dantrolene-treated hearts during experiments. The mean duration of all VF episodes combined was shorter in dantrolene-treated hearts (226.55 \pm 79.7 s versus 574.4 \pm 144.3 s, *P*=0.03). Sustained VF (VF lasting \geq 60 s) was observed in 67% (16/24) of episodes in controls in comparison with 29% (10/34) in dantrolene-treated hearts (*P*=0.03, repeated measures logistic regression).

Dantrolene Increased Alternans Threshold and Decreased Diastolic Calcium Leak From RyR2 After VF

SCaE in diastole occurred after first or second VF episode (\pm isoproterenol [0.3 µmol/L]) in nontreated rabbit hearts detected as elevation in calcium fluorescence before the initiation of the first spontaneous beat after the termination of pacing (Figure 3). SCaE was either absent at baseline (before VF \pm isoproterenol) or was associated with short amplitude relative to the pacing beats in hearts in both groups (0.015 \pm 0.01 arbitrary unit at 200 ms PCL, 0.027 \pm 0.01 arbitrary unit at 180 ms PCL). SCaE amplitude significantly (*P*=0.0004) increased from baseline after 2 VF episodes in controls from 0.02 \pm 0.01 arbitrary unit to 0.12 \pm 0.02 arbitrary unit at 200 ms PCL. As shown in Figure 3, the administration of 20 µmol/L

dantrolene almost prevented or significantly decreased the SCaE amplitude after VF.

With the subsequent induction of VF episodes, calcium alternans emerged at longer PCL in the control hearts. Calcium alternans emerged at shorter PCL in dantrolene-treated hearts with a 10% decrease from 195 \pm 9.5 ms PCL at baseline to 173 \pm 6.7 ms post-VF in comparison with an 11% increase in controls from 215 \pm 20 ms to 235 \pm 8 ms (*P*=0.05; Figure 3).

Increased Spatiotemporal Organization of Calcium Waves During VF in Dantrolene-Treated Hearts

Number of calcium wave fronts was analyzed as a surrogate for spatial organization of calcium waves during VF (ie, fewer wave fronts translates into higher organization of VF). Number of calcium wave fronts at 4 minutes of VF was significantly lower in dantrolene-treated hearts than in controls (2.2 ± 0.06 versus 2.7 ± 0.07 waves per frame, P=0.02). See details in Figure I in the online-only Data Supplement.

Mechanism of Antiarrhythmic Effect of Dantrolene on VF-Induced Arrhythmias

Rapid pacing (at 10 Hz) induced a large increase in the number of spontaneous sarcomere-shortening events per second on termination of pacing (pacing-induced rapid activation).



Figure 3. Dantrolene reduced diastolic spontaneous calcium elevation amplitude and increased calcium alternans threshold in rabbit hearts. **1**, Representatives of calcium transients and diastolic spontaneous calcium elevations (SCaEs; blue arrows) in dantrolene-treated (**top**) and control (**bottom**) rabbit hearts. Dantrolene infusion significantly decreased or completely abolished diastolic SCaE (red arrow). Amplitude of SCaE was calculated by measuring the difference between the onset of SCaE and the onset of the first spontaneous calcium transient and is normalized to the amplitude of calcium transients during pacing. **2**, In control hearts, after 2 VF episodes (±soproterenol), the amplitude of SCaE significantly increased from baseline at 200 ms and 180 ms PCL. In dantrolene-treated hearts, the amplitude of SCaE was significantly lower than that of controls, and dantrolene significantly mitigated the amplitude of SCaEs. ****P*=0.004 vs control (for treatment and time interaction). At 200 and 180 ms PCL after 2 VF episodes, SCaE amplitude was 0.12 AU and 0.17 AU, respectively, in controls and 0.02 AU and 0.02 AU in dantrolene-treated hearts (*P*=0.001 and *P*=0.0003, respectively), **3. Top**, Recording of calcium signals was acquired after 30 s of continuous pacing at 200 ms CL after 1st and 2nd VF episodes. Calcium amplitude alternans emerged after 1st VF. On dantrolene infusion, alternans was significantly mitigated. **3. Bottom**, Calcium alternans threshold was not statistically different between groups at baseline (215 vs 195 ms, *P*=0.008 vs control [post-VF]). ISO indicates isoproterenol; PCL, pacing cycle length; and VF, ventricular fibrillation.

Dantrolene treatment reduced the rate of spontaneous shortening observed after pacing cessation (P<0.0001; Figure 4), thereby enhancing the return of infrequent spontaneous activity observed in myocytes before pacing.

Mathematical Modeling of Purkinje Fibers

Consistent with the presence of delayed after-depolarizations in the isolated myocytes, delayed after-depolarizations were routinely observed in the myocardium of the rabbit heart in the model. These delayed after-depolarizations developed into action potentials, with several firing before spontaneously stopping as sarcoplasmic reticulum calcium became depleted. In the organ-scale model, delayed after-depolarizations arising in the myocardium were always subthreshold, a consequence of the electrotonic coupling of the tissue. Conversely, delayed after-depolarizations occurring in the Purkinje system did precipitate propagating action potentials, which were transmitted into the myocardium via anterograde activation. These observations were true for all stochastic distributions studied.

Setting the level of RyR2 block in the model was based on the rabbit experimental calcium measurements. With a 12% reduction in RyR2 conduction, the minimum pacing cycle length necessary for calcium alternans was reduced from 240 ms to 185 ms, consistent with the experimental measurements (from 195 to 173 ms; Figure 4). Adding

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Figure 4. Dantrolene mitigated the pacing-induced rapid activation in isolated cardiomyocytes and prevented pacing-induced delayed after-depolarizations in Purkinje fibers in computer simulations. Comparison of spontaneous pacing-induced rapid activation in isolated cardiomyocytes. **1**, All cells were treated with 100 nmol/L of isoproterenol and in a subgroup, 20 µmol/L dantrolene was added to the cells. **1**, **Top**, ECG tracings recorded from a cardiomyocytes. Isoproterenol was added before fast pacing (black arrow). Cardiomyocytes were burst paced at 10 Hz for 10 s. Dantrolene was added after the first episode of fast pacing (blue arrow), and the protocol was repeated. The duration of pacing-induced rapid activation (dashed lines) was significantly shorter in dantrolene-treated cardiomyocytes, and the cycle length of spontaneous activation increased significantly faster than in controls. (*P*<0.0001). **2**, Linear regression fit of time to return of prepacing spontaneous activation after termination of fast pacing and comparison of the linear regression slope of pacing-induced rapid activity time-interval curve in cardiomyocytes. **3A**, Intracellular calcium traces of rabbit ventricular myocytes model paced at 220 ms CL. A 12% reduction in RyR2 conductance (bottom trace) eliminates alternans. **B**, Transmembrane voltage recording for simulated canine Purkinje cell and ventricular myocyte after pacing at 350 ms CL. Delayed after-depolarization–induced action potentials (') and subthreshold depolarizations (**) occur spontaneously in both models after pacing is stopped. **C**, Activation map of first postpause propagated response resulting from a delayed after-depolarization. White asterisks denote sites of delayed after-depolarization formation in the PS leading to propagated action potentials. CL indicates cycle length; and ISO, isoproterenol.

dantrolene to the Purkinje cell ionic model completely abolished the appearance of ectopic action potentials after a pace-and-pause procedure. A 12% reduction in RyR2 conductance did not abolish delayed after-depolarizations developing into action potentials, but a 47% reduction did. In the ventricular model, dantrolene prevented the appearance of action potentials arising from delayed after-depolarizations. Even at a level insufficient to stop action potential formation in isolated ventricular myocytes, no action potentials spontaneously developed from the myocardium. Applying dantrolene to the Purkinje system alone was sufficient to inhibit delayed after-depolarization–induced action potentials.

Discussion

We have demonstrated that, in an in vivo model of cardiac arrest due to VF and CPR, dantrolene significantly improves survival by mitigating the time-dependent increasing disorganization of VF, enhancing defibrillation success, enhancing the achievement of the return of spontaneous circulation, and preventing postdefibrillation hemodynamic compromise. Dantrolene also led to fewer refibrillations and, in addition, did not alter refractoriness. The drug also improved spatiotemporal organization of calcium waves during VF in ex vivo rabbit hearts. Most importantly, in rabbit hearts, dantrolene increased the calcium alternans threshold and significantly reduced RyR2-dependent diastolic calcium leak. Furthermore, dantrolene-treated rabbit hearts were more resistant to VF induction and refibrillation. Taken together, these findings suggest a potential novel strategy of using dantrolene for improving resuscitation outcomes by normalizing VF-induced calcium dysregulation.

Dantrolene has been safely used in clinical settings for years as a stabilizer of skeletal muscle RyR1. Recently, dantrolene was shown to bind to RyR2, which plays a crucial role in calcium cycling and cardiac contractility.^{12,13,17} Dantrolene has been shown to directly bind to domain 601 to 620 of RyR2 in failing cardiomyocytes and to stabilize the interdomain interaction within the channel and significantly improve the sarcoplasmic reticulum calcium reserve.^{12,13,17}

Dantrolene Enhances Defibrillation Success

Dantrolene infusion during CPR was associated with earlier defibrillation success and shortened time to ROSC in this study. This can be related, in part, to the improved organization of VF after dantrolene infusion. In the control group, the organization of VF signals significantly decreased over time during VF. Dantrolene prevented the time-dependent disorganization of VF signals and enhanced defibrillation. Additionally, there was a significant increase in the organization of calcium wave fronts in isolated rabbit hearts after treatment with dantrolene in comparison with controls. Dantrolene-treated rabbit hearts were less susceptible to the induction of VF or the transformation of VF to ventricular tachycardia in <60 s.

VF is associated with [Ca]²⁺, overload and the derangement of cardiac calcium cycling.⁷ [Ca]²⁺, overload can contribute to sustaining VF and failed defibrillations.^{18,19} The long duration of calcium transients and the disorganized calcium cycling during VF has been proposed as a possible mechanism for long-duration VF.20 At 5 minutes of long-duration VF, calcium transients have been shown to become longer and more disorganized throughout the epicardium and endocardium with significant calcium alternans.20 It has also been demonstrated that [Ca]²⁺, and the changes in calcium transient amplitude can indeed affect APD during VF and promote wave break.²¹ In fact, [Ca]²⁺, can act as both a driver and modulator for the wave break.22 Therefore, agents that modulate calcium cycling can potentially enhance defibrillation success.¹⁹ For example, administration of cariporide (a Na+/H+ exchanger blocker) is shown to improve myocardial electric and mechanical stability after VF and CPR by indirectly reducing the VF-induced [Ca]²⁺, overload.²³

We observed a significant reduction in calcium wave fronts during VF after treatment with dantrolene, which translates to increased spatial organization of calcium waves during VF. This may further explain the transition of VF to ventricular tachycardia or self-termination of VF in several dantrolene-treated rabbit hearts. It has been proposed that non–voltage-gated calcium currents (mainly RyR2 and SERCA2 activity) contribute to the maintenance of VF.²⁴ These local calcium release events during VF can change APD and promote wave breaks.²⁴ Additionally, spontaneous calcium release and the formation of calcium sinkholes after defibrillation shocks have been proposed to decrease the chance of successful defibrillation shocks.²⁵ The stabilization of RyR2 and the suppression of these local calcium release events during VF can potentially break the vicious cycle of long-duration VF and [Ca]²⁺, overload and enhance defibrillation success as proposed by other groups.^{5,24,25} Purkinje fiber activation is reported to be responsible for postshock arrhythmias and unsuccessful shocks after VF both in experimental^{25,26} and computer modeling²⁷ studies. In our study, dantrolene suppressed after-depolarizations and triggered activity in Purkinje fibers (presumably by suppressing the afore-mentioned diastolic SCaEs) and, therefore, prevented APD formation and the propagation in Purkinje fibers after fast pacing and VF (Figure 4). Therefore, the suppression Purkinje fiber activation during VF by dantrolene can further explain the enhanced defibrillation success and reduced number of shock-resistant VFs in the dantrolene group in our in vivo study.

Dantrolene Improved Hemodynamic Outcomes Postdefibrillation

Among survivors of the in vivo pig experiments, dantrolene-treated pigs had significantly higher systolic and diastolic BP than controls. The rise in BP was specifically evident in the first 10 minutes postdefibrillation with a peak at 5 minutes. ROSC was achieved significantly earlier, and myocardial stunning was ameliorated in dantrolene-treated pigs.

This improvement in hemodynamics by dantrolene can be explained by 2 mechanisms: (1) the shorter time to defibrillation in dantrolene group, and (2) dantrolene's effect on cardiac calcium handling and the improvement in contractility by normalizing the VF-induced calcium dysregulation.

The rise in catecholamine levels postdefibrillation²⁸ can result in significant stimulation of β-receptors in cardiomyocytes, impair calcium cycling by affecting ion channels such as RyR2, and result in diastolic calcium leak. Calcium leaks from sarcoplasmic reticulum in diastole will compromise the sarcoplasmic reticulum calcium reserve for subsequent beats resulting in a decrease in cardiac contractility.^{10,29} The same cascade of events can happen in VF and diminish cardiac contractility postdefibrillation. [Ca]2+, overload caused by VF and dysfunction in cardiac calcium cycling can diminish contractility.^{4,6} Similarly, we have shown that significant diastolic calcium leak from RyR2 develops after VF (+isoproterenol) in isolated rabbit hearts. These VF-induced calcium leaks from RyR2 coupled with the afore-mentioned rise in β-adrenergic stimulation postdefibrillation may underlie the lower pressure postdefibrillation in control pigs. Dantrolene infusion nearly abolished diastolic calcium leak in rabbit hearts post-VF and therefore can also explain the significant improvement of hemodynamics in the dantrolene group in comparison with controls.

Other studies have shown similar inotropic effects of dantrolene in the presence of adrenergic stimulation. Dantrolene was shown to improve the force frequency relationship in failing human myocardium by enhancing cardiac contractility in the presence of sympathetic stimulation.³⁰ The effect was more pronounced in the presence of higher isoproterenol concentrations. The peak systolic $[Ca]^{2+}_{i}$ was not different between the 2 groups, suggesting that the enhanced inotropic response to isoproterenol is due to the modulation of the diastolic concentration of cytosolic calcium by dantrolene.³⁰

It was also reported that dantrolene protects cardiac tissue against injury induced by excess β -receptor stimulation.³¹ More recently, it was shown that dantrolene restores cardiac contractility by suppressing diastolic calcium leak from RyR2.¹³

Mechanism of Reduction of Refibrillations by Dantrolene

Dantrolene-treated pigs experienced fewer episodes of refibrillation. Additionally, the inducibility and sustainability of subsequent VF was significantly attenuated in dantrolene-treated rabbit hearts. To evaluate whether the restoration of cardiac calcium cycling by dantrolene attributed to the observed antiarrhythmic effect, we evaluated the effect of dantrolene on 2 mechanisms by which dysfunctional calcium cycling results in arrhythmias and refibrillation in particular: (1) diastolic calcium leak, delayed after-depolarizations, and triggered activity; and (2) calcium alternans and reentry.

Dantrolene infusion significantly reduced the pacinginduced rapid activation of isolated mice cardiomyocytes in the presence of isoproterenol, which demonstrates a direct effect of dantrolene as an antiarrhythmic agent. Based on our modeling of Purkinje cells, dantrolene specifically terminated delayed after-depolarizations arising from Purkinje fibers as a result of VF-like activation (fast pacing). Delayed after-depolarizations and triggered activity have been proposed as a possible mechanism of refibrillation. Where there is continuous calcium leak from sarcoplasmic reticulum either as a result of the hyperphosphorylation of calcium channels by Ca²⁺/calmodulin-dependent protein kinase II and protein kinase A activity (adrenergic stimulation) or genetic disorders of the channels, the resultant diastolic elevation in $[Ca]^{2+}$ has been shown to activate compensatory mechanisms such as Na/ Ca exchanger. Na/Ca exchanger activation during the repolarization phase can then induce delayed after-depolarizations that eventually leads to arrhythmias.8,29 The same concept can be applied to VF and refibrillation, because VF is associated with a significant increase in Ca2+/calmodulin-dependent protein kinase II activity (due to fast activation) and adrenergic stimulation.28,32

Previous studies have shown that these diastolic elevations in [Ca]²⁺, depend on RyR2 activity (leak) and can result in delayed after-depolarizations and triggered activity in the endocardium specifically in Purkinje fibers.^{16,26,33} Similarly, we found that VF-like activation promotes delayed after-depolarizations in our model in cardiomyocytes and Purkinje fibers, but only delayed after-depolarizations arising from Purkinje fibers could generate APDs propagating throughout the myocardium. Specifically in isolated myocytes, VF simulation resulted in spontaneous sarcomere shortenings. These sarcomere-shortening events are consistent with the appearance of delayed after-depolarizations, which are indicative of calcium overload. Dantrolene abolished these delayed after-depolarizations at the Purkinje level, which can be explained by the effect of dantrolene on the prevention, or at least the mitigation of diastolic SCaE as observed in our experimental rabbit study. These findings further support our hypothesis that rapid activation of the heart (whether cardiomyocytes or Purkinje fibers) results in excess activation of RyR2 and eventually leads to delayed after-depolarizations and refibrillations. The stabilization of RyR2 by dantrolene provided antiarrhythmic benefits and reduced refibrillation. Almost all refibrillations in the in vivo study were triggered by ectopic beats (triggered activity), which supports the theory that after-depolarizations cause refibrillation and that dantrolene reduces these refibrillations by modulating calcium cycling and suppressing delayed after-depolarizations at the Purkinje level.

The lower incidence of successfully induced or sustained refibrillation in dantrolene-treated rabbit hearts suggests that dantrolene directly plays a role in the regulation of calcium cycling during VF, and, by restoring RyR2 function, increased the calcium alternans threshold. It also prevented delayed after-depolarization formation at the Purkinje level after VF simulation. Refibrillations likely result from nonorganized spontaneous sarcoplasmic reticulum calcium release in the form of nonorganized calcium waves in the Purkinje system, and dantrolene provided antiarrhythmic benefits by suppressing these arrhythmogenic calcium waves in the Purkinje system.

Study Limitations

One of the limitations to this study is that healthy pigs with no underlying heart failure or coronary disease were studied. Although the evidence is strong for direct interaction of dantrolene with RyR1, RyR3, and RyR2, we cannot rule out the possible effect of dantrolene on other channels. However, we analyzed the changes in QT intervals and early repolarization pattern before and after VF as a surrogate for assessing the potassium channels involved in repolarization, and we found no impact.

We used an equivalent of the adult dose of defibrillation energy in the in vivo study, but the pigs weighed within the pediatric range (\approx 30 kg). Thoracic impedance of pigs and humans is different, and higher defibrillation energy levels are usually required for pigs in comparison with humans. However, the relatively high energy level might have had an impact in the defibrillation outcomes and should be taken into account.

Another limitation to this study is that different species were used for the in vivo and ex vivo studies. The interspecies variability in VF dynamics and defibrillation might affect the results and might limit the ability to extrapolate the findings from 1 model to the other.

Additionally, it is not clear whether the findings from these models would relate to resistant VF in humans. Therefore, the results should be interpreted cautiously in this context.

Conclusion

Dantrolene infusion during VF facilitates successful defibrillation, improves hemodynamics postdefibrillation, decreases refibrillation, and thus improves survival after cardiac arrest without promoting arrhythmias. The effects are mediated by enhancing calcium cycling and, at the Purkinje level, preventing arrhythmogenic calcium leak in diastole and suppressing triggered activity caused by rapid activation and sympathetic stimulation. Dantrolene might prove to be a useful adjunctive treatment for the management of sudden cardiac arrest due to VF.

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Disclosures

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CLINICAL PERSPECTIVE

Despite decades of research in resuscitation, survival from sudden cardiac arrest due to ventricular fibrillation (VF) is still very low. The use of various antiarrhythmic agents has not resulted in the improvement in survival to discharge. Studying the molecular pathways and ion channels contributing to the maintenance of VF has become an area of interest in resuscitation research. In this study, we have demonstrated that targeting the VF-induced impairment of cardiac calcium cycling due to ryanodine receptor-2 dysfunction, by administration of dantrolene, significantly enhances defibrillation and facilitates the return of spontaneous circulation. The strategy also improved cardiac contractility postdefibrillation and reduced refibrillations. Modeling and computer simulations demonstrated that these effects of dantrolene are mostly mediated through its effects on Purkinje fibers. These findings suggest a novel therapeutic strategy in the management of sudden cardiac arrest due to VF. Dantrolene has been shown to be a safe drug and has been used in emergency settings such as malignant hyperthermia. Given its ease of delivery and rapid effect, dantrolene might prove to be an adjunctive treatment to cardiopulmonary resuscitation and defibrillation in sudden cardiac arrest due to VF.

SUPPLEMENTAL MATERIAL

Supplemental Materials and Methods

In-vivo swine model

Following endotracheal intubation anesthesia was maintained by continuous administration of Isoflurane (2% mixed with 100% O2). Animals were mechanically ventilated (Ohio ventilator R.A.E. Technologies, Inc. Ontario) at 21 breaths/min and Endotracheal Tube CO2 (ETCO2) was continuously measured using CO2MO Plus monitoring system (Novametrix Medical Systems). Two self-adhesive defibrillation pads were attached to the lateral aspects of the chest wall for defibrillation and for ECG monitoring. (Lifepak 12, Medtronic-PhysioControl, Redmond, WA)

- Electrophysiological and Hemodynamic Monitoring

Femoral arteries and veins were cannulated and an EP catheter (EP Technologies Inc, Sunnyvale, CA) was placed in the right ventricle to enable pacing and to induce VF. Two micro manometer-tipped Millar catheters (Millar Instruments, Inc, Houston, TX) were placed in abdominal aorta and right atrium. The pressure and ECG signals were recorded at 1000Hz with a 0.05Hz high pass and 500Hz low pass filter by custom designed recording system and acquisition software. (Acqui2, Cartesian Labs , Toronto, On.)

- In-Vivo Model Definitions

Re-fibrillation was defined as recurrence of VF after at least 5 beats of a non-VF rhythm following defibrillation. "Initial survivor" was defined as animals with successful

defibrillation of initial VF to pulsatile normal sinus rhythm. Time to Return of Spontaneous Circulation (ROSC) was calculated from successful defibrillation until Systolic BP (sBP) \geq 60 mmHg in the abdominal aorta was observed. Sustained ROSC was defined as maintenance of sBP 260 mmHg by the end of 30-min post-defibrillation. Ventricular ERP was measured at baseline and at 20 minutes into recovery (if any) via the S1-S2 stimulation method. Surface ECG signals at 4 min of VF and at 7 min of VF were extracted to analyze for VF organization. A Spatio-temporal index of VF organization (Regularity Index) was used.¹ Dominant frequency was measured using the Power spectral density of the ECG signals recorded from the surface ECG. Each power spectral density was scanned between 5.5 and 15 Hz and the frequency associated with the highest energy component was extracted as the dominant frequency. Regularity index was defined as the ratio of the power at the dominant frequency to the total power. The power at the dominant frequency was calculated by summing the power values at the highest peak and its adjacent values (fixed band of 1 Hz). The total power was calculated as the sums over the range of 5.5-15 Hz. Values vary between 0 (disorganized) to 1 (highly organized).

Ex-vivo Rabbit Langendorff model

All animals were anesthetized with sodium pentobarbital (35 mg/kg), aorta was attached to the Langendorff apparatus and retrogradely perfused with 37.5°C oxygenated Tyrode solution with albumin 80 mg/L dissolved in de-ionized water equilibrated with 95% O2 and 5% CO2. Simultaneous optical mapping of epicardial surface of isolated hearts for calcium and voltage was performed using 0.5 mg Rhod2-AM and 10 microM RH237.

Blebbistatin at 10 microM was added to Tyrode solution to block cardiac contractions. Hearts were excited at 530 nm and light was emitted through a Dichroic mirror at 630 nm. The wavelength below 630 nm was emitted through a 585 interference filter to measure $[Ca^{2+}]i$ and wavelength above 630 was emitted through a 715 nm short pass filter to measure membrane potential.

Since induction of VF in healthy isolated rabbit hearts is not always successful, we created a specific criterion to compare inducibility and sustainability of VF between groups. As a result, we only analyzed rabbit hearts in which we could successfully induce 2 episodes of VF each lasting for 4 min with 5 min recovery in between. Dantrolene was added during VF (at 1st min of VF). We evaluated the vulnerability of rabbit hearts to refibrillations in the control and dantrolene groups by inducing subsequent VF episodes by burst pacing. Inducibility (VF lasting for ≥ 10 sec) and Sustainability (VF lasting ≥ 60 sec) and total duration of these subsequent re-fibrillation episodes was measured and compared between dantrolene and controls. Additionally, hearts were paced at 250, 220, 200, 180 and 160 msec Pacing Cycle Length (PCL) for 30 seconds after the first and second VF episodes.

Diastolic Spontaneous Calcium Elevation (SCaE) was detected during the diastolic pause after termination of pacing (after 30 sec of continuous pacing to reach steady state) as the rise in calcium sensitive fluorescence during the diastolic pause and before initiation of the first spontaneous beat after termination of pacing. The SCaE amplitude was measured and normalized to calcium transient amplitude during pacing and is presented as Arbitrary Units (AU). The protocol to measure SCaE amplitudes was adopted from Lee, Y. et al.²

Isolated Cardiomyocytes

To determine the effect of dantrolene on isolated myocytes, cells were isolated from 8 weeks old CD1 mouse left ventricles and VF was simulated by rapid pacing. Cells were kept in Tyrode solution, containing (mM): 140 NaCl, 4 KCl, 1 MgCl2, 0.5 CaCl2, 10 HEPES, 10 D-glucose, pH 7.35 with NaOH. Experiments were performed at room temperature in Tyrode's solutions with Ca^{2+} elevated to 1.6mM. The protocols used are shown in figure 4. The cells were then field stimulated at 10Hz for 7~15 seconds after being exposed to the drug for >50 sec. After termination of stimulation, spontaneous activity typically increased gradually. The duration of the post-pacing spontaneous sarcomere shortening.

Determining differential effects on myocytes and Purkinje cells by mathematical modeling

To determine the differential effect of post-VF re-fibrillation on myocytes compared to Purkinje fibers, a pace and pause protocol was employed in a realistic whole heart cardiac model. A finite element model of the ventricles and Purkinje system was used as previously developed.^{3, 4} For the ventricular myocardium, the Hund-Rudy canine ionic model ⁵ was implemented, and for the Purkinje system, the Li-Rudy model of the canine Purkinje cells ⁶ was used. To generate delayed after-depolarization in the myocardium, store overload induced Calcium release was added to the model by setting a junctional

sarcoplasmic reticulum threshold of 3 mM, above which RyR2 receptors opened to release Calcium; to model this phenomenon, we used the same approach described by Heiiman et al.⁷ Inward rectifier current was reduced by 50% and maximal Na⁺/Ca²⁺ exchanger density was increased by 50%, since afterdepolarizations induced by store overload-induced calcium release do not elicit action potentials in the unmodified model; these changes are among those known to occur in failing ventricular myocytes. As described elsewhere³, delayed after-depolarizations in the Purkinje system model were elicited by decreasing the RyR2 release time constant by 66% (to 2 msec), and increasing sensitivity to luminal Calcium by 75%. Using these values, isolated and Purkinje and myocardial cells displayed approximately equal propensity for after-depolarization generation. Effects of dantrolene were modeled by a decrease in RyR2 conductance based on matching a rabbit ionic model⁸ to the ex-vivo optical Calcium transient measurements, and by blocking RyR3⁹ which was only present in the Purkinje cell model. To determine initial conditions, single cell models were pre-paced for 100 beats at a cycle length of 350 msec. Furthermore, [Ca²⁺]i in the Purkinje cell junctional sarcoplasmic reticulum was set to 2.5 mM. A stochastic approach was used to model tissue-scale heterogeneity of sarcoplasmic reticulum state; this was necessary to prevent all cells from behaving as an ensemble (i.e. simultaneously firing delayed afterdepolarization). The time constant of RyR2 was distributed normally with an average of 2 msec and a variance of 20%, while in the myocardium, the store overload-induced calcium release threshold had a mean of 3 mM and a variance of 20%. Two pacing pulses were applied to the apex 400 msec apart and then ensuing activity was monitored.

Supplemental Results

- Ex-vivo Rabbit Langendorff

In isolated rabbit hearts the changes in APD80 (from 0 to 80% repolarization) after VF was similar between dantrolene-treated and non-treated groups. ($10\%\pm6\%$ reduction from baseline in controls vs. $4\%\pm7\%$ reduction in dantrolene group at 200 msec PCL, P=0.65)

- Increased Spatio-temporal organization of Calcium waves during VF in dantrolene treated hearts

Using phase maps of voltage and Calcium signals during VF, we compared the spatiotemporal organization of VF between control and dantrolene-treated groups. Number of wavefronts in these phase maps during VF was used as a surrogate for quantifying the organization of VF. (i.e. fewer number of wavefronts translates into higher organization of VF) Administration of dantrolene during VF significantly reduced the number of calcium wavefronts and improved organization of calcium waves at 4 min of VF compared to time 0. (Figure 1-supp)

Although the number of calcium wavefronts at time 0 of the 2^{nd} VF was significantly lower compared to time 0 of the 1^{st} VF in both groups (P=0.024 for the time effect, repeated measures analysis), Calcium waves at time 0 of VF were more organized in dantrolene group as implied by the significantly (P=0.027) lower mean number of calcium wavefronts at time 0 of the 2^{nd} VF in dantrolene-treated hearts (2±0.12 waves per frame recording) compared to controls (2.49±0.1 waves per frame). There was a significant dissociation between the number of voltage and calcium wavefronts after 4 min of VF in control hearts. (1.76 ± 0.37 APD waves per frame vs. 2.7 ± 0.1 Calcium waves per frame, P=0.02) However, in dantrolene treated hearts, there was no significant difference between the number of voltage and Calcium wavefronts. (2.73 ± 0.66 vs. 2.11 ± 0.07 , P=0.28)



Figure 1-Supp. Increased spatiotemporal organization of Calcium waves during VF in dantrolene-treated rabbit hearts

(1) Representation of Calcium and voltage phase maps of epicardial surface of rabbit hearts in control and dantrolene groups. Black arrows: Collision of 2 wavefronts in the center of the mapped area of a control heart. In dantrolene-treated hearts phase maps of calcium signals revealed a more organized pattern with mostly one wave sweeping across the mapped area during VF (white arrows) resembling a VT-like activation pattern. (2) A representative of experimental protocol. Optical recording of calcium and voltage signals to acquire phase maps was performed during the first 4 sec after successful induction of 1st VF and at 4 min of VF and right after induction of the 2nd VF.. (3)-left and middle: During the first few seconds after induction of 1st VF, there was no difference in the number of voltage or calcium wavefronts between groups indicating similar spatial organization of VF. Mean number of calcium wavefronts per frame did not change after 4 min of VF compared to baseline (0 min) in controls but significantly decreased after infusion of dantrolene during VF. (P=0.032 for drug and time interaction) The change in the mean number of APD wavefronts at 4 min (from time 0) was similar in both groups. (P=0.16 for drug and time interaction). (3) right: After dantrolene infusion in dantrolene group or saline in the controls, 2nd VF was induced. Calcium waves were significantly more organized in dantrolene-treated hearts compared to controls.

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Dantrolene Improves Survival After Ventricular Fibrillation by Mitigating Impaired Calcium Handling in Animal Models

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