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

Running title: Zamiri et al.; Improving survival from VF by dantrolene

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Abstract

Background—Resistant ventricular fibrillation, re-fibrillation and diminished myocardial contractility are important factors leading to poor survival following cardiac arrest. We hypothesized dantrolene improves survival following VF by rectifying calcium dysregulation caused by VF.

Methods and Results—VF was induced in 26 Yorkshire pigs for 4 min. CPR was then commenced for 3 min and dantrolene or isotonic saline was infused at the onset of CPR. Animals were defibrillated and observed for 30 min. To study the effect of VF on calcium handling and its modulation by dantrolene, hearts from 14 New-Zealand rabbits were Langendorff-perfused. Inducibility of VF following dantrolene administration was documented. Optical mapping was performed to evaluate diastolic spontaneous calcium elevations (SCaE) as a measure of cytosolic calcium leak. Sustained Return of Spontaneous Circulation (ROSC) (sBP≥60mmHg) was achieved in 85% of dantrolene group compared to 39% of controls. (P=0.02) ROSC was achieved earlier in dantrolene-treated pigs after successful defibrillation. (21±6 sec vs. 181±57 sec in controls, P=0.005) Median number of re-fibrillation episodes was lower in dantrolene group (0 vs. 1, P= 0.04). In isolated rabbit hearts, 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 leak in the form of SCaEs. Administration of 20μM dantrolene significantly decreased SCaE amplitude vs. controls. (0.024±0.013 vs. 0.12±0.02 AU (200 msec CL), P=0.001)

Conclusions—Dantrolene infusion during CPR facilitates successful defibrillation, improves hemodynamics post-defibrillation, decreases re-fibrillation and thus improves survival following cardiac arrest. The effects are mediated through normalizing VF-induced dysfunctional calcium cycling.

Key words: resuscitation, cardiac arrest, ventricular fibrillation, ryanodine receptor, calcium channel
Introduction

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. Cardiopulmonary Resuscitation CPR and defibrillation are the mainstays of immediate management; however, despite significant progress in CPR techniques in last decades, mortality from VF has remained unacceptably high, with 23.8% surviving to hospital admission and only 7.6% survival to hospital discharge.

Shock-resistant VF, decreased cardiac contractility post-defibrillation (myocardial stunning) and recurrence of VF (Ventricular Re-fibrillation) are main challenges faced during CPR and increase morbidity and mortality.

Studies of ion channel blockers have shown little or no benefit in experimental and clinical VF. However, VF is associated with significant impairment in cardiac calcium cycling and results in Intracellular Calcium ([Ca^{2+}]i) overload. Dysfunction in [Ca^{2+}]i handling and calcium overload during VF has been proposed to cause myocardial stunning post-defibrillation and trigger re-fibrillation by activating compensatory mechanisms such as the Na^{+}/Ca^{2+} Exchanger and causing after-depolarizations. Failed defibrillation or re-fibrillation leads to more ischemia, worsening the calcium overload. Stabilizing cardiac calcium cycling may be able to mitigate this vicious cycle, enhance defibrillation and thus improve survival following VF.

Ryanodine Receptor-2 (RyR2) is the major calcium release channel expressed in the sarcoplasmic reticulum and plays a crucial role in cardiac contractility. Recently, it has been proposed that increased calcium leak from RyR2 results in progression of heart failure and triggers VF. Stabilization of RyR2 function is shown to be protective against cardiac arrhythmias in animal models of Catecholaminergic Polymorphic Ventricular Tachycardia.
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 and improve cardiac function and prevent arrhythmogenesis in various animal models of heart failure.\textsuperscript{11-13} We hypothesized that 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 re-fibrillation. We further hypothesized these effects of dantrolene are mediated via its effects on stabilizing cardiac calcium cycling that results from VF induced dysfunction of \([\text{Ca}^{2+}]_{\text{i}}\) handling.

Methods

In-vivo Swine Model

Healthy 10-12 weeks-old Yorkshire pigs with weight ranging between 27-34kg (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 supplemental materials. After initial stabilization, VF was induced by burst pacing (10V of 60Hz current for 2 seconds) and left untreated for 4 minutes. Then, chest compression was started using a pneumatic device (Lucas, Jolife AB, Lund, Sweden) at 100 compressions/min and manual ventilation at 6 breaths/min using 5-6 liters/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 hyperthermia\textsuperscript{14} and other animal models.\textsuperscript{15} After 7 min of VF, defibrillation was attempted at 150J. If defibrillation was unsuccessful, CPR was immediately restarted and defibrillation was again attempted at 200J after 2 min (with subsequent defibrillation attempts at 300J\textrightarrow{}360J\rightarrow{}360J\rightarrow{}360J\rightarrow{}360J in case of 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 min to assess the outcome and occurrence of re-fibrillations. Re-fibrillation 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 2.4-4.5Kg were used. (n=14) Details on the model and measurements are provided in the supplemental materials. VF was induced by burst pacing for 30 sec at 60 Hz and 10V and hearts were continuously perfused during VF. At 1 min of VF, a single dose of dantrolene (20\(\mu\)M) or isotonic saline was infused in the bubble trap. VF was monitored for 4 min and then defibrillated at 5J. Five more episodes of fibrillation-defibrillation were tried on each heart with 3 min recovery time (in sinus rhythm) in between. VF duration for each VF episode was 4 min (or shorter in 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 beat-to-beat difference of more than 10\% in calcium wave amplitude. Alternans threshold was defined as the longest Pacing Cycle length (PCL) at which alternans emerged. Diastolic Spontaneous [Ca\textsuperscript{2+}]i 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, Y. et al.\textsuperscript{16}
Isolated Cardiomyocytes and Mathematical Modeling

A detailed description of the isolated cardiomyocyte study and mathematical modeling is provided in the supplemental materials. Briefly, isolated cardiomyocytes were paced at 10 Hz to mimic the high activation during VF in the presence of 100 nM 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 APD formation in Purkinje system and the whole heart was assessed.

Statistical Analysis

Time to ROSC, time to successful defibrillation and onset of re-fibrillation were analyzed using 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 using two way repeated-measures ANOVA with pairwise analysis using Sidak's correction for multiple comparisons. Comparison of ordinal variables (number of re-fibrillations, number of defibrillation attempts) and continuous variables in unpaired groups was performed using Wilcoxon Mann-Whitney test. 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 using Fisher exact test. P ≤ 0.05 was considered statistically significant. For repeated measures analysis between groups, P value for treatment and time interaction is provided unless otherwise stated. For pairwise comparisons, the adjusted P value for multiple comparisons are provided. All statistical analysis were performed using Stata 11.1 (Stata Corp LP) and SPSS 17.
Results

Dantrolene Increased Survival Following VF

VF was successfully induced in all 26 animals. Pulsatile normal sinus rhythm immediately post-defibrillation (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 compared to 39% (5/13) in the Controls. (P=0.02) (Table 1)

Dantrolene Organized VF Signals And Facilitated Defibrillation

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

In all animals combined (survivors and non-survivors) 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 min of VF (3 min after initiation of CPR) 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, dominant frequency after 7 min of VF was significantly (P=0.047) lower in dantrolene treated pigs compared to controls. (9.2±0.62 Hz and 11.2±0.61 Hz respectively)

Hemodynamic Outcomes Following CPR

While successful defibrillation was achieved significantly (P=0.006) earlier in dantrolene-treated pigs, time to ROSC was also shorter in dantrolene group. ROSC (sBP≥60 mmHg) was achieved
after 21±6 sec post-defibrillation in dantrolene group vs. 181±57 sec in controls. (Figure 2)

Systolic and Diastolic pressure during post-defibrillation period in dantrolene group were significantly higher than controls. (P=0.0014 and P=0.0017 respectively) (Figure 2) By the end of the experiments, mean sBP and dBP was 85 [95% CI 77-93] and 61 [95% CI 54-68] mmHg in survivors in dantrolene group and 66 [95% CI 53-79] and 46 [95% CI 37-55] mmHg in Controls respectively.

In order 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 min. (n=8 in dantrolene group and n=4 in controls). Time to achieving a systolic pressure ≥60 mmHg was shorter in dantrolene group. (26±8 sec vs. 93±48 sec in controls, P=0.06); Systolic and diastolic pressures were significantly higher in dantrolene-treated pigs compared to their control peers during the 30 min post-defibrillation period. (P=0.0016 and P=0.002 for the treatment effect)

**Dantrolene Decreased Re-fibrillation And Improved Outcomes Following Re-fibrillation**

Median number of re-fibrillation episodes was significantly lower in the dantrolene group compared to controls. Re-fibrillation occurred in 2 scenarios: when post-defibrillation rhythm was normal sinus rhythm with pulsatile rhythm (initial survivors) and when the post-defibrillation rhythm was either pulseless electrical activity or asystole. Since the first scenario is more clinically relevant, we analyzed re-fibrillation in more detail in initial survivors of the VF. (Table 1) All re-fibrillations in the dantrolene group were terminated with the first defibrillation attempt and with no impact on hemodynamic parameters and survival afterwards. Re-fibrillations were triggered by PVCs in 96% of re-fibrillation episodes (24/25). Only in 1 case re-fibrillation
was preceded by sustained VT.

**Dantrolene Did Not Alter Refractoriness**

There was no significant difference in post-defibrillation effective refractory period between dantrolene and controls. (215±9 msec vs. 206±4 msec, P=0.47) In dantrolene group, QT interval at baseline and post-defibrillation was 432±48 msec and 334±83 msec while in controls, the corresponding values were 442±50 msec and 325±6 msec respectively. The change in post-defibrillation QT intervals vs. baseline was not significant between control and dantrolene groups. (P=0.35)

**Ex-vivo Rabbit Protocol**

**Dantrolene-treated Hearts Were Resistant To VF and Re-fibrillations**

After initial induction of 2 VF episodes, dantrolene-treated hearts were more resistant to re-fibrillation episodes. In controls, 20 (83%) of 24 attempts resulted in VF (VF lasting ≥10 sec) compared to 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 VT in dantrolene-treated hearts. Additionally, spontaneous re-fibrillation occurred in 4 control hearts and in none of dantrolene-treated hearts during experiments. The mean duration of all VF episodes combined was shorter in dantrolene-treated hearts. (226.55±79.7sec vs. 574.4±144.3 sec, P=0.03) Sustained VF (VF lasting ≥60 sec) was observed in 67% (16/24) of episodes in controls compared to 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**

Spontaneous Calcium Elevation (SCaE) in diastole occurred after first or second VF episode (+/-
Isoproterenol (0.3μM) in non-treated rabbit hearts detected as elevation in calcium fluorescence before the initiation of the first spontaneous beat after termination of pacing. (Figure 3) SCaE was either absent at baseline (before VF +/- isoproterenol) or was associated with short amplitude relative to the pacing beats in hearts in both groups. (0.015±0.01AU at 200msec PCL, 0.027±0.01AU at 180msec PCL) SCaE amplitude significantly (P=0.0004) increased from baseline after 2 VF episodes in controls from 0.02±0.01 AU to 0.12±0.02 AU at 200 msec PCL. As shown in figure 3, administration of 20μM dantrolene almost prevented or significantly decreased the SCaE amplitude after VF.

With 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 10% decrease from 195±9.5 msec PCL at baseline to 173±6.7 msec post-VF compared to 11% increase in controls from 215±20 msec to 235±8 msec. (P=0.05)(Figure 3)

Increased Spatio-temporal Organization of Calcium Waves During VF in Dantrolene-treated Hearts

Number of calcium wavefronts was analyzed as a surrogate for spatial organization of calcium waves during VF. (i.e. fewer wavefronts translates into higher organization of VF) Number of calcium wavefronts at 4 min of VF was significantly lower in dantrolene-treated hearts compared to controls. (2.2±0.06 vs. 2.7±0.07 waves per frame, P=0.02). See details in supplemental data. (Figure 1-supp)

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 upon termination of pacing (pacing-induced rapid activation).

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 prior to 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 msec to 185 msec, consistent with the experimental measurements (195 to 173 msec). (Figure 4) Adding 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 achievement of return of spontaneous circulation and preventing post-defibrillation hemodynamic compromise. Dantrolene also led to fewer re-fibrillations 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 calcium alternans threshold and significantly reduced RyR2 dependent diastolic calcium leak. Furthermore, dantrolene-treated rabbit hearts were more resistant to VF induction and re-fibrillation. 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 is shown to bind to RyR2, which plays a crucial role in calcium cycling and cardiac contractility. Dantrolene is shown to directly bind to domain 601-620 of RyR2 in failing cardiomyocytes and stabilize the interdomain interaction within the channel and significantly improve the sarcoplasmic reticulum calcium reserve.

**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 in part related to improved organization of VF after dantrolene infusion. In the control group, 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 organization of calcium wavefronts in isolated rabbit hearts after treatment with dantrolene compared to controls. Dantrolene-treated rabbit hearts were less susceptible to induction of VF with most VF episodes resulted in self termination of VF or transformation of VF to VT in less than 60 sec.

VF is associated with [Ca\(^{2+}\)]\(_i\) overload and derangement of cardiac calcium cycling.\(^7\) [Ca\(^{2+}\)]\(_i\) overload can contribute to sustaining VF and failed defibrillations.\(^18,19\) Long duration of calcium transients and disorganized calcium cycling during VF has been proposed as a possible mechanism for Long Duration VF (LDVF).\(^20\) At 5 min of LDVF, calcium transients are shown to become longer and more disorganized throughout epicardium and endocardium with significant calcium alternans.\(^20\) It has also been demonstrated that [Ca\(^{2+}\)]\(_i\) and changes in calcium transient amplitude can indeed affect APD during VF and promote wave break.\(^21\) In fact, [Ca\(^{2+}\)]\(_i\) can act as both driver or modulator for wavebreak.\(^22\) 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 electrical and mechanical stability following VF and CPR by indirectly reducing the VF-induced [Ca\(^{2+}\)]\(_i\) overload.\(^23\)

We observed a significant reduction in calcium wavefronts 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 VT 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 maintenance of VF.\(^24\) These local calcium release events during VF can change APD and promote wavebreaks.\(^24\) Additionally, spontaneous calcium release and formation of calcium sinkholes after defibrillation shocks are proposed to
decrease the chance of successful defibrillation shocks.\textsuperscript{25} Stabilization of RyR2 and suppressing these local calcium release events during VF can potentially break the vicious cycle of LDVF and [Ca$^{2+}$]i overload and enhance defibrillation success as proposed by other groups.\textsuperscript{5, 24, 25}

Purkinje fiber activation is reported to be responsible for post-shock arrhythmias and unsuccessful shocks following VF both in experimental\textsuperscript{25, 26} and computer modeling\textsuperscript{27} studies. In our study, dantrolene suppressed after-depolarizations and triggered activity in Purkinje fibers (presumably by suppressing the aforementioned diastolic SCaEs) and therefore, prevented APD formation and propagation in Purkinje fibers after fast pacing and VF (figure 4). Therefore, suppressing Purkinje fiber activation during VF by dantrolene can further explain the enhanced defibrillation success and reduced number of shock-resistant VF in dantrolene group in our in-vivo study.

\textbf{Dantrolene Improved Hemodynamic Outcomes Post-Defibrillation}

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

This improvement in hemodynamics by dantrolene can be explained by two mechanisms:

1. Shorter time to defibrillation in dantrolene group
2. Dantrolene’s effect on cardiac calcium handling and improving contractility by normalizing the VF-induced calcium dysregulation

The rise in catecholamine levels post-defibrillation\textsuperscript{28} can result in significant stimulation of beta-receptors in cardiomyocytes, impair calcium cycling by affecting ion channels such as RyR2 and result in diastolic calcium leak. Calcium leak from sarcoplasmic reticulum in diastole will compromise the sarcoplasmic reticulum calcium reserve for subsequent beat resulting in...
decrease in cardiac contractility. The same cascade of events can happen in VF and diminish cardiac contractility post-defibrillation. [Ca^{2+}]_i overload caused by VF and dysfunction in cardiac calcium cycling can diminish contractility. Similarly, we have shown that significant diastolic calcium leak from RyR2 develops after VF (+Isoproterenol) in isolated rabbit hearts. These VF-induced calcium leak from RyR2 coupled with the aforementioned rise in beta-adrenergic stimulation post-defibrillation may underlie the lower pressure post-defibrillation 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 compared to controls.

Other studies have shown similar inotropic effects of dantrolene in the presence of adrenergic stimulation. Dantrolene was shown to improve force frequency relationship in failing human myocardium by enhancing cardiac contractility in the presence of sympathetic stimulation. The effect was more pronounced in the presence of higher isoproterenol concentrations. The peak systolic [Ca^{2+}]_i was not different between the two groups suggesting that the enhanced inotropic response to isoproterenol is due to modulation of the diastolic concentration of cytosolic calcium by dantrolene. It was also reported that dantrolene protects cardiac tissue against injury induced by excess beta receptor stimulation. More recently, it was shown that dantrolene restores cardiac contractility by suppressing diastolic calcium leak from RyR2.

**Mechanism of Reduction of Re-fibrillations by Dantrolene**

Dantrolene-treated pigs experienced fewer episodes of re-fibrillation. Additionally, inducibility and sustainability of subsequent VF was significantly attenuated in dantrolene-treated rabbit hearts. To evaluate whether 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 re-fibrillation in particular: 1) Diastolic Calcium leak, delayed after-depolarizations and Triggered activity, 2) Calcium alternans and re-entry

Dantrolene infusion significantly reduced the pacing-induced 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 has been proposed as a possible mechanism of re-fibrillation. Where there is continuous calcium leak from sarcoplasmic reticulum either as a result of the calcium channels’ hyperphosphorylation by CaMKII and PKA activity (adrenergic stimulation) or genetic disorders of the channels, the resultant diastolic elevation in [Ca^{2+}]i has been shown to activate compensatory mechanisms such as Na/Ca exchanger. Na/Ca exchanger activation during repolarization phase can then induce delayed after-depolarizations that eventually leads to arrhythmias. The same concept can be applied to VF and re-fibrillation since VF is associated with significant increase in CaMKII activity (due to fast activation) and adrenergic stimulation.

Previous studies have shown these diastolic elevations in [Ca^{2+}]i are dependent on RyR2 activity (leak) and can result in delayed after-depolarizations and triggered activity in the endocardium specifically in Purkinje fibers. 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 Purkinje level and this can be explained by the effect of dantrolene on preventing or at least mitigating 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 re-fibrillations.

Stabilization of RyR2 by dantrolene provided antiarrhythmic benefits and reduced re-fibrillation. Almost all re-fibrillations in the in-vivo study were triggered by ectopic beats (triggered activity) which supports the theory of after-depolarizations causing re-fibrillation and that dantrolene reduces these re-fibrillations by modulating calcium cycling and suppressing delayed after-depolarizations at Purkinje level.

The lower incidence of successfully induced or sustained re-fibrillation in dantrolene-treated rabbit hearts, suggests that dantrolene directly played role in regulation of calcium cycling during VF and by restoring RyR2 function, increased calcium alternans threshold. It also prevented delayed after-depolarization formation at the Purkinje level after VF simulation. Re-fibrillations likely result from non-organized spontaneous sarcoplasmic reticulum calcium release in the form of non-organized 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. Though evidence is strong for direct interaction of dantrolene...
with RyR1, RyR3 and RyR2 we can’t rule out the possible effect of dantrolene on other channels. However, we analyzed the changes in QT intervals and ERP before and after VF as a surrogate for assessing the potassium channels involved in repolarization and 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 (~30 Kg). Thoracic impedance of pigs and humans is different and higher defibrillation energy levels are usually required for pigs compared to 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 inter-species variability in VF dynamics and defibrillation might affect the results and might limit the ability to extrapolate the findings from one 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 post-defibrillation, decreases re-fibrillation and thus improves survival following cardiac arrest without promoting arrhythmias. The effects are mediated by enhancing calcium cycling and at 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 management of sudden cardiac arrest due to VF.

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**References:**


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Table 1. Summary of hemodynamic and re-fibrillation parameters in dantrolene and Control groups

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Dantrolene</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Successful defibrillation*</td>
<td>85% (11/13)</td>
<td>100% (13/13)</td>
<td>0.24</td>
</tr>
<tr>
<td>Time to defibrillation* (sec)</td>
<td>Median: 351±37</td>
<td>Median: 231±19</td>
<td>0.006</td>
</tr>
<tr>
<td>Total time in VF‡</td>
<td>181±57</td>
<td>21±6</td>
<td>0.005</td>
</tr>
<tr>
<td>Shock-resistant VF (&gt;2 defibrillation attempts)</td>
<td>54% (7/13)</td>
<td>8% (1/13)</td>
<td>0.015</td>
</tr>
<tr>
<td>Maximum energy level</td>
<td>252±24J</td>
<td>181±16J</td>
<td>0.02</td>
</tr>
<tr>
<td>Total energy level</td>
<td>699±186</td>
<td>333±119</td>
<td>0.04</td>
</tr>
<tr>
<td>Re-fibrillation Incidence**</td>
<td>71% (5/7)</td>
<td>27% (3/11)</td>
<td>0.08</td>
</tr>
<tr>
<td>Number of Re-fibrillation episodes</td>
<td>1.5±0.6</td>
<td>0.5±0.4</td>
<td>0.04</td>
</tr>
<tr>
<td>Time to onset of Re-fibrillation (sec)</td>
<td>421±301</td>
<td>55±11</td>
<td>0.22</td>
</tr>
<tr>
<td>Sustained ROSC after Re-fibrillation</td>
<td>40% (2/5)</td>
<td>100% (3/3)</td>
<td>0.35</td>
</tr>
<tr>
<td>Mean number of shocks to terminate each Re-fibrillation episode</td>
<td>2.2±0.5</td>
<td>1±0</td>
<td>0.05</td>
</tr>
</tbody>
</table>

** Metabolic Parameters

- **PH (prior to defibrillation)**
  - Control 7.45±0.02
  - Dantrolene 7.4±0.07

- **PaO2 (prior to defibrillation)**
  - Control 130±51
  - Dantrolene 189±30

- **PaCO2 (prior to defibrillation)**
  - Control 36±3.2
  - Dantrolene 36±2.7

*: 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 post-defibrillation until sBP of >60mmHg was achieved. ‡: Total time in VF includes the duration of initial VF plus all re-fibrillations observed during the experiment. ** Data only includes initial survivors of VF with normal sinus rhythm with any detectable BP immediately post-defibrillation. Analysis of time to ROSC, time to defibrillation and time to re-fibrillation was performed using Log-rank (Mantel-Cox) test for survival analysis. ROSC: Return of Spontaneous Circulation
Figure Legends:

Figure 1. Comparison of temporal organization of VF between groups (in-vivo experiments). (1) Regularity Index (RI) was measured at baseline (T0), 4 min of VF (prior to CPR) and 7 min of VF (3 min after drug infusion, prior to defibrillation). (sample surface ECG tracing recorded from a control and a dantrolene-treated pig at 4 min of VF (before CPR) and at 7 min 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 control group, VF organization consistently deteriorated during 7 min of VF. (P=0.031). RI at 7 min of VF was 0.69 (95% CI 0.6-0.78) and 0.55 (95% CI 0.49-0.6) in dantrolene and Control group respectively.(P=0.004) There was no statistical difference between RI at T0 and 4 min 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 min in dantrolene and control group respectively, P=0.96) (3) Dotplots demonstrating the % Change in dominant frequency (top) and regularity index (bottom) of VF after 7 min of VF from baseline (T0); dominant frequency increased by 35% in control group and by 15% in dantrolene group. (P=0.16) VF became significantly more disorganized during 7 min of VF in controls. (31% reduction in VF organization (RI) from vs. 11% in dantrolene group (P=0.006).

Figure 2. Hemodynamic parameters (sBP, dBP, heart rate) throughout the experiment in dantrolene and Control group in survived pigs. (1)The data presented during the post-defibrillation period only includes survivors (ROSC at 30 min after termination of VF). Left: changes in systolic BP, Right: changes in diastolic BP; VF0: BP during first min of VF, VF4: BP...
during the 4th min of VF, CPR0: BP during first min of CPR, CPR3: BP during the 3rd min of CPR, sBP was specifically higher at 5 min post-defibrillation in those treated with dantrolene (103±7.2 mmHg (95% CI 87-120 mmHg) vs. 58±5.8 mmHg (95% CI 44-73 mmHg), P=0.002), Both sBP and dBP were significantly higher during the post-defibrillation 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 to ROSC (sBP≥60 mmHg) between groups after defibrillation. ROSC was achieved significantly earlier in dantrolene group during post-defibrillation period compared to the control group.(P=0.005, Survival analysis with Log-rank (Mantel-Cox) test)(3) Changes in heart rate post-defibrillation in survivors - The heart rate was lower in dantrolene group but was not statistically different from controls.(P=0.12).

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 (SCaE) (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. One arbitrary unit (AU) equals the mean amplitude of calcium transients during pacing. (2) In control hearts, after 2 VF episodes (+/- Isoproterenol) the amplitude of SCaE significantly increased from baseline at 200 msec and 180 msec pacing CL. 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), **: P=0.008 vs. control
(for treatment and time interaction). At 200 and 180 msec 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 sec of continuous pacing at 200 msec CL after 1st and 2nd VF episodes. Calcium amplitude alternans emerged after 1st VF. Upon dantrolene infusion, alternans was significantly mitigated. (3)-bottom: Calcium alternans threshold was not statistically different between groups at baseline, (215 vs. 195 msec, P=0.48) however, After 2 VF episodes, alternans emerged at shorter PCL (173 msec vs. 235 msec in controls) in dantrolene-treated hearts. (**: P=0.008 vs. control (post-VF)).

**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 nM of Isoproterenol and in a subgroup, 20μM of 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 sec. 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 compared to controls. (P<0.0001) (2) Linear regression fit of time to return of pre-pacing spontaneous activation after termination of fast pacing and comparison of the linear regression slope of pacing-induced rapid activity time-interval curve in cardiomyocytes. (3)-A) Intracellular calcium traces of rabbit ventricular
myocytes model paced at 220 msec 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 msec CL. delayed after-depolarization-induced action potentials (*) and subthreshold depolarizations (**) occur spontaneously in both models after pacing is stopped. C) Activation map of first post-pause 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.
Figure 1

1. VF → CPR (+Dantrolene) → Shock
   - Time 0 → 4 min → 7 min

2. Control vs. Dantrolene
   - Regularity Index (RI) = a/(a+b)
   - P = 0.0004
   - P = 0.23
   - P = 0.031

3. % Change in dominant frequency
   - P = 0.16
   - Control vs. Dantrolene
Figure 2
Figure 3

(1) Diagram showing the effects of Dantrolene and Saline on ventricular fibrillation (VF) and isoproterenol (ISO) induced arrhythmias. The graph illustrates the sequence of events leading to VF and the impact of Dantrolene on the second VF.

(2) Graph showing the mean amplitude of SCaE (AU) at different time points: baseline, post-VF1, and post-VF2. The data is compared between control and Dantrolene groups.

(3) Graph illustrating the calcium alternans threshold (ms CL) before and after Dantrolene treatment. The comparison shows a statistically significant reduction in the threshold post-treatment. **P = 0.008**.
Figure 4

(1) Pseudo-ECG

Isoproterenol (100nM)

Dantrolene (20 μM)

Burst pacing (10 sec)

(Pacing-induced rapid activation)

(2) Pacing-induced Spontaneous activity CL (ms)

ISO (100 nM) + Dantrolene

ISO (100 nM)

P<0.0001

Time (from termination of pacing) (s)

(3) A

Control

B

Pacrine

12% Rythm block

C

not yet activated

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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) ≥60 mmHg in the abdominal aorta was observed. Sustained ROSC was defined as maintenance of sBP≥60 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. 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 re-fibrillations in the control and dantrolene groups by inducing subsequent VF episodes by burst pacing. Inducibility (VF lasting for \(\geq 10\) sec) and Sustainability (VF lasting \(\geq 60\) 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 activity was quantified by measured the sustained presence of 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 Heijman et al.\(^7\) Inward rectifier current was reduced by 50% and maximal Na\(^+\)/Ca\(^{2+}\) 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 elsewhere \(^3\), 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 model\(^8\) to the ex-vivo optical Calcium transient measurements, and by blocking RyR3\(^9\) 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, [Ca\(^{2+}\)]\(_i\) 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 after-depolarization). 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%±6% reduction from baseline in controls vs. 4%±7% 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 2nd VF was significantly lower compared to time 0 of the 1st 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 2nd 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.
References