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An invited review following the *Soujinkai Young Investigator Award*: Stabilization of RyR2 Maintains Right Ventricular Function, Reduces the Development of Ventricular Arrhythmias, and Improves Prognosis in Pulmonary Hypertension

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Abstract Right ventricular (RV) dysfunction and its linked arrhythmias play a crucial role in determining the prognosis of pulmonary arterial hypertension (PAH). Our paper aimed to explore the potential protective effects of direct pharmacological intervention in the RV muscle using dantrolene (DAN), a stabilizer of the cardiac ryanodine receptor (RyR2), against RV dysfunction and arrhythmia in a rat model of monocrotaline (MCT)-induced PAH. To induce PAH, male 8-week-old Sprague-Dawley rats received MCT injections. The study also assessed the induction of ventricular tachycardia (VT) by catecholamines, examining RyR2-mediated Ca^{2+} release properties in isolated cardiomyocytes. Additionally, a pulmonary artery-banding model was established to evaluate the independent effects of chronic pressure overload on RV morphology and function. In the MCT-induced PAH rat model, findings revealed RV hypertrophy, dilation, and functional decline, resulting in 0% survival rate two months post-MCT induction. Conversely, chronic DAN treatment demonstrated improvements in these RV parameters and an 80% increase in survival. Furthermore, chronic DAN treatment prevented the dissociation of calmodulin from RyR2, inhibiting Ca^{2+} sparks and spontaneous Ca^{2+} transients in MCT-induced hypertrophied RV cardiomyocytes. Epinephrine induced VT in over 50% of rats with MCT-induced PAH, while chronic DAN treatment achieved complete suppression of VT. The paper concludes that stabilizing RyR2 with DAN holds promise as a novel therapeutic approach against the development of RV dysfunction and fatal arrhythmias associated with PAH.

Key words: calmodulin, ryanodine receptor, pulmonary arterial hypertension, dantrolene, ventricular arrhythmia

Introduction

Pulmonary arterial hypertension (PAH) is a condition characterized by a grim prognosis and a rising prevalence.[1] Despite recent advancements in prognosis with triple therapy involving phosphodiesterase-5 inhibitors, endothelin antagonists, and prostaglandins,[2-5] patients with advanced right ventricular (RV) failure continue to face poor outcomes globally[6,7] and in Japan.[8] The absence of an effective means to directly address RV dysfunction resulting from chronic pressure overload contributes to the persistent poor prognosis of PAH.

In previous studies using a tachycardia-induced canine heart failure mode[1,9] transverse aortic coarctation (TAC) mouse model,[10,11] and catecholaminergic polymorphic ventricular tachycardia (CPVT) type R2474S knock-in (KI) mouse model,[12-14] we illustrated that Ca^{2+} leakage stems from a reduction in calmodulin (CaM) binding affinity for ryanodine receptor (RyR2) due to defective interdomain interaction between N-terminal and central domains, known as domain unzipping,[12,15,16] leading to heart failure and fatal arrhythmias. Recently, through screening 25 mutant peptides in the CaM-binding domain of RyR2 (3584-3603), we identified a single amino acid mutation (V3599K) significantly enhancing the binding affinity of CaM for RyR2. Subsequently, the RyR2 V3599K KI mouse was developed, and mating these mice with R2474S CPVT-associated mice suppressed catecholamine-stimulated CaM dissociation, eliminating exercise-induced ventricular tachycardia (VT).[17] Moreover, in the V3599K KI mouse, TAC failed to induce cardiac hypertrophy and heart failure in the left ventricle (LV), suggesting that the dissociation of CaM from RyR2 followed by Ca^{2+} leakage is crucial for pressure overload-induced hypertrophy and heart failure development.[18]

Thus, cardiac hypertrophy emerges as an adverse prognostic factor for pressure overload-induced heart failure, even during the early compensatory phase. Addressing cardiac hypertrophy at the cellular level from the onset of pressure overload may enhance the prognosis of pressure overload-induced heart failure beyond reliance on vasodilatation. In this context, our research has demonstrated that dantrolene (DAN), a drug used for malignant hyperthermia treatment, elevates the binding affinity of CaM for RyR2 and suppresses Ca^{2+} leakage by directly binding to the N-terminal region (amino acid [a.a.] 601-620),[16,19] thereby preventing intracellular signaling leading to cardiac hypertrophy[18,20] and arrhythmias induced by pressure overload.[11]

This study investigates whether DAN-mediated suppression of pressure overload-induced hypertrophy improves RV function, diminishes fatal arrhythmias, and enhances the prognosis in rats with monocrotaline (MCT)-induced PAH.

DAN demonstrated a notable improvement in prognosis by preventing RV dysfunction in rats with MCT-induced PAH

In the MCT-treated group, all rats succumbed within 2 months of MCT induction. However, the prognosis significantly ameliorated in the MCT + DAN group, as depicted in Figures 1A and 1B. Postmortem examination of 5 MCT-treated rats revealed pleural effusion in all cases, indicating right heart failure as a potential cause of death. In the MCT group, the RV expanded with a concurrent decrease in the left ventricle (LV), a phenomenon that was prevented in the MCT + DAN group (Figure 1C). While RV pressure increased in the MCT group, it showed a moderate decrease in the MCT + DAN group (Figure 1D). Additionally, DAN treatment successfully suppressed the increase in RV weight observed in the MCT group. The LV weight remained consistent across all groups (Figure 1E). The MCT group exhibited significantly higher collagen levels in the RV wall compared to the sham group, a effect that was notably suppressed in the MCT + DAN group (Figure 1E). It is noteworthy that DAN had no discernible effect on any of the measured parameters in sham animals.

DAN successfully prevented inducible VT in rats with MCT-induced PAH

The injection of caffeine plus epinephrine consistently triggered sustained bigeminy and VT in the MCT group, while such

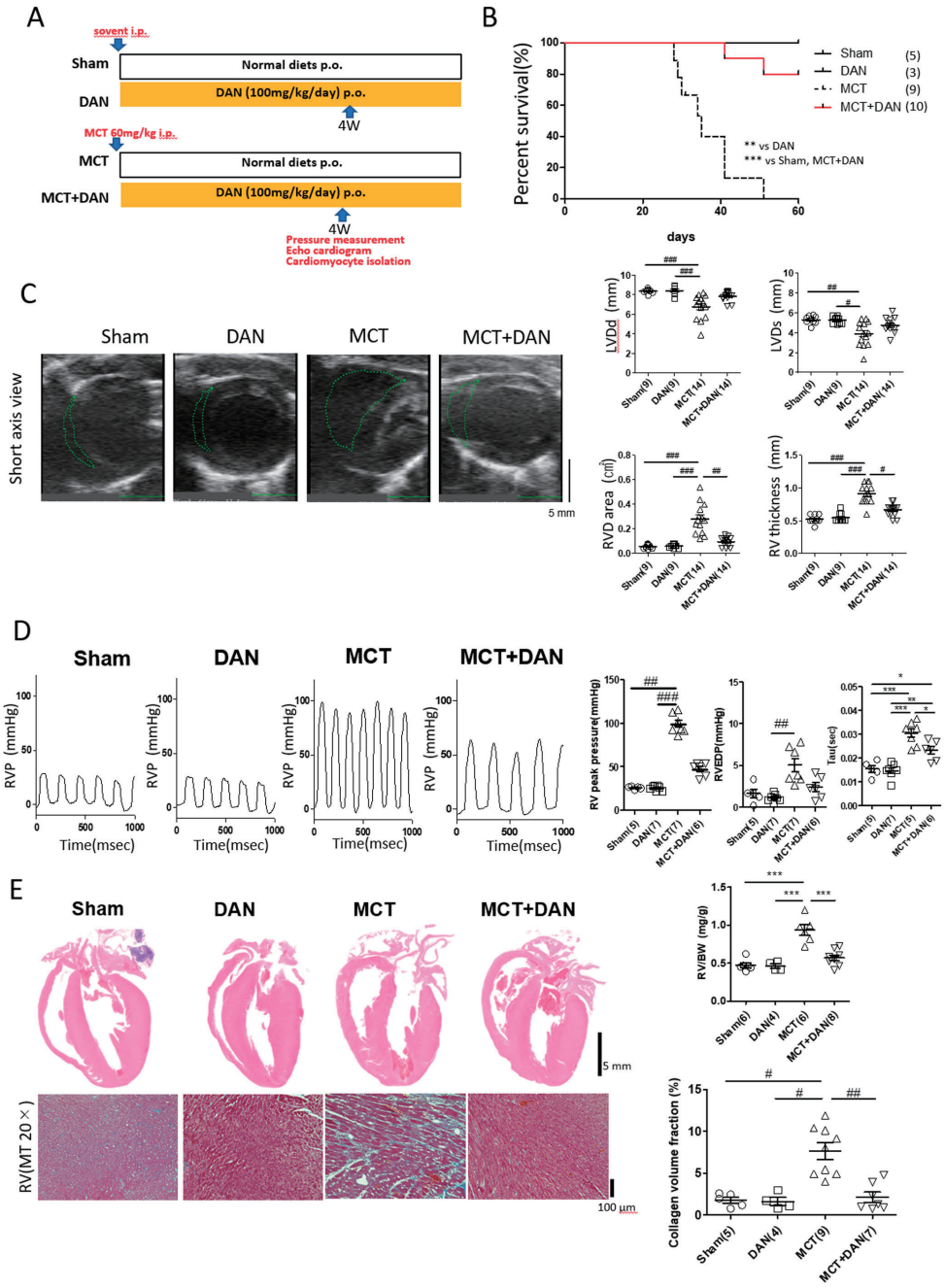

Fig. 1 Research design, survival analysis, echocardiograms, RV pressure curves, and stained tissues. A: Study design of MCT-induced PAH rat model. B: A Kaplan-Meier survival analysis. Values in parentheses indicate the number of rats. C: Representative echocardiograms and summarized data (n = 9-14 as indicated). D: Representative RV pressure curves and summarized data (n = 5-7 as indicated). E: Representative images of long-axis sections of the hearts and the HE- or MT-stained RV tissues. Summarized RV and LV weight data (n = 4-8 as indicated) are shown in the right upper panel. Fibrotic areas of the RV free wall obtained from MT-stained RV tissue are shown in the right lower panel (n = 4-9; 3 images of 20× objective from each rat are analyzed). **P < .01, ***P < .001 (log-rank test) in panel B. **P < .01, ***P < .001 (analysis of variance with a post hoc Tukey's test) in panels C, D, and E. #P < .05, ##P < .01, ###P < .001 (Kruskal-Wallis test with a post hoc Dunn's test) in panels C, D, and E. This figure is a quote with modification from the award-winning paper[25] under permission.

arrhythmic episodes were notably absent in the MCT + DAN group (Figure 2A). Details of the observed arrhythmogenic episodes are succinctly outlined in Figure 2B.

The mechanism underlying the stabilizing effect of DAN on RyR2

In recent years, significant advancements in cryo-electron microscopy have enabled the analysis of the three-dimensional structure of ryanodine receptor 2 (RyR2) at nearly atomic resolution.[21,22] This progress revealed the three-dimensional existence of the zipping interface, which links the N-terminal domain and the central domain across different subunits. The domain linkage occurs within a relatively narrow range (N-terminal domain: 1-220; central domain: 2250-2500) and is in proximity to the calmodulin (CaM) binding site (3583-3603). This suggests that domain unzipping leads to the dissociation of CaM due to surrounding structural changes, and vice versa.

The N-terminal-central domain interaction interface forms a hinge at four places, and the spatial distance changes only slightly during physiological opening and closing.[22] This implies that this region is precisely the "key point" for stabilizing the tetrameric structure. If these domain interaction interfaces are unzipped, it is reasonable to speculate that the tetrameric structure loosens, resulting in dilation of the central channel pore and causing Ca^{2+} leakage. The binding site of dantrolene (DAN) (amino acids 601-620) is three-dimensionally located near both the zipping interface and the CaM binding region. This suggests that DAN may structurally stabilize the CaM-RyR2 binding and domain zipping,[9,12,16] thereby stabilizing the channels.

Remarkably, the binding site of DAN (amino acids 601-620) is adjacent to that of K201 (JTV519), which was initially discovered to have a stabilizing effect on RyR2[23] and later identified to bind at RyR2 residues 2114-2149.[24] Consequently, both drugs may share a similar mechanism in correcting defective CaM-RyR2 interactions in diseased hearts (Figure 3).

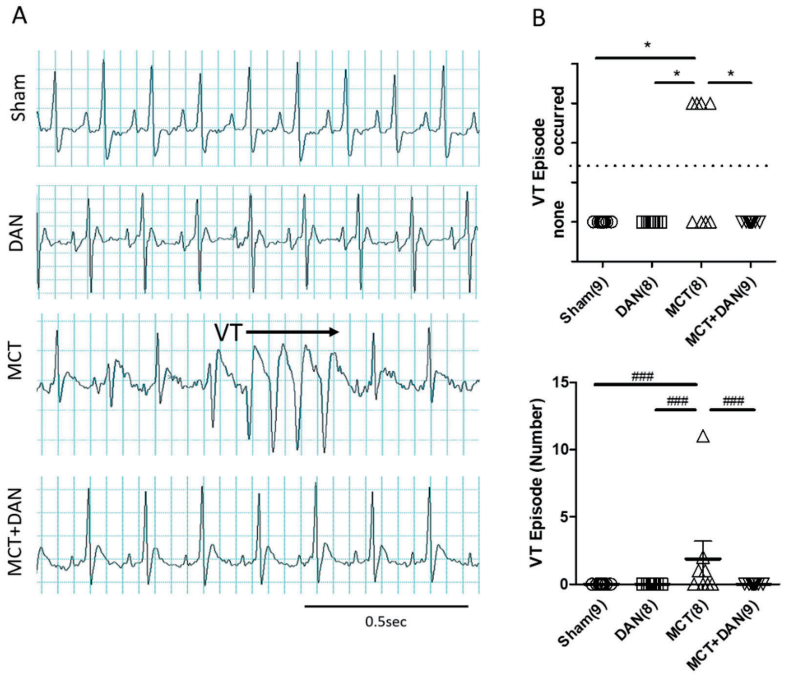

Fig. 2 Inhibitory effect of DAN on epinephrine-provoked ventricular arrhythmia in rats with MCT-induced PAH. A: Representative electrocardiograms upon stimulation by epinephrine (1 mg/kg of body weight, intraperitoneally). B: Summarized data of 8-9 rats as indicated. *P < .05 (χ^2 test with a post hoc Ryan's method). This figure is a quote with modification from the award-winning paper[25] under permission.

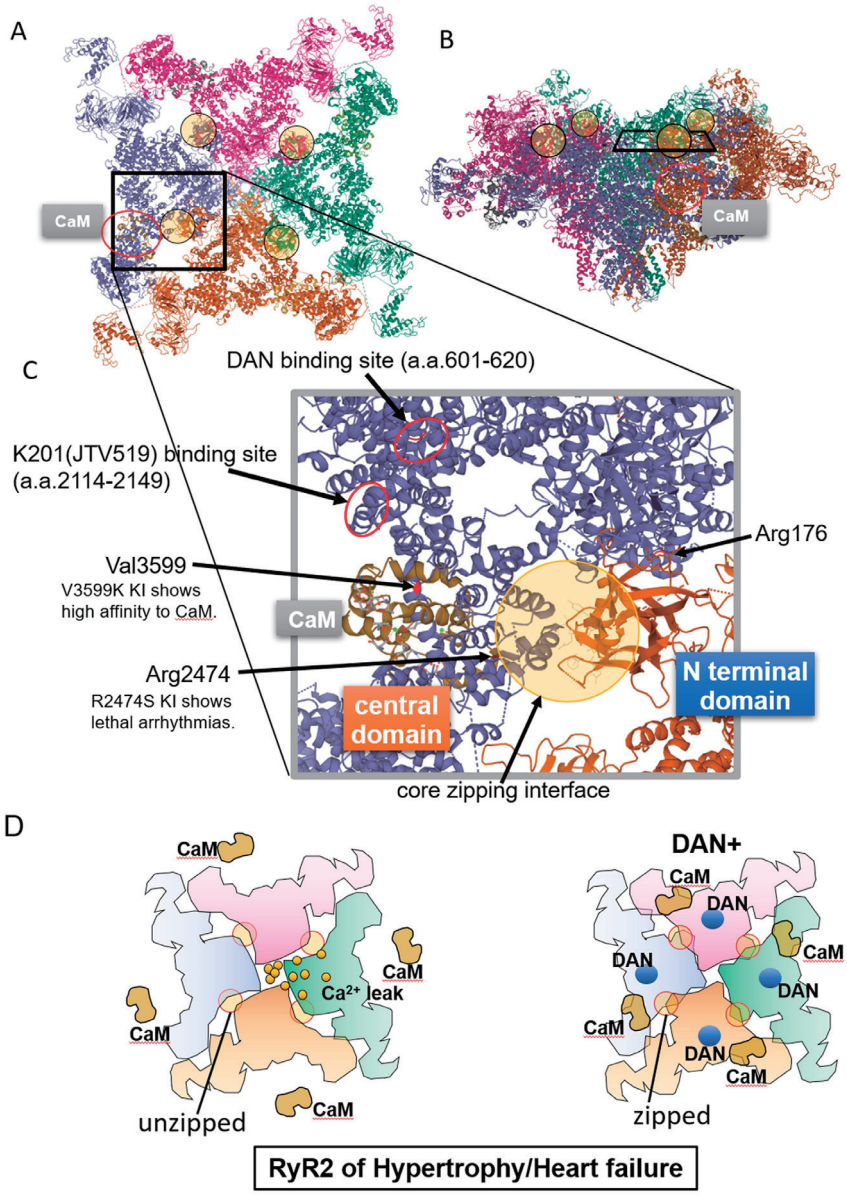

Fig. 3 Tight intersubunit interaction at the core zipping interface and CaM binding are essential to maintain the conformational stability of RyR2 (3-dimensional structure was referred from PDBID:6JV2 [21]). A: Plan view of the RyR2 tetramer. B: Side view. C: Enlarged image near the core zipping interface. The interface between N-terminal (a.a. 1-220; orange) and central (a.a. 2250-2500; blue) domains forms an intersubunit interaction. The CaM binding domain (a.a. 3583-3603) is close to this zipping core. DAN (a.a. 601-620) is located near both the zipping interface and the CaM binding region. Interestingly, the binding site of DAN (a.a. 601-620) is just adjacent to that of another RyR2 stabilizer, K201 (JTV519) (a.a. 2114-2149). D: Our modified "zipping-unzipping" hypothesis based on higher-order structural analysis. In hypertrophied and/or heart failure cardiomyocytes, domain unzipping occurs at the core zipping interface, followed by CaM dissociation and Ca^{2+} leakage. DAN enhances the interaction between subunits by binding near the core zipping interface ("unzipped" to "zipped"), prevents CaM dissociation, and suppresses Ca^{2+} leakage in diseased hearts.[9,12,16] Interestingly, the binding site of DAN is just adjacent to another RyR2 stabilizer of K201 (JTV519),[23,24] suggesting that both agents stabilize the tetrameric structure of RyR2 through the same mechanism. This figure is a quote with modification from the award-winning paper[25] under permission.

Conclusions

In summary, the elevated RV pressure in PAH triggers Ca^{2+} leakage from RyR2, initiating cardiac hypertrophy and contributing to a poor prognosis characterized by RV dysfunction and fatal arrhythmias. DAN directly binds to RyR2, providing structural stabilization to the channel. This mechanism effectively suppresses the progression of these pathological events, ultimately leading to an improved prognosis. Consequently, DAN holds promise as a novel and potent therapeutic agent for patients with PAH.

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Conflict of Interest

The authors declare no conflict of interest.

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