

Short- and long-term effects of dronedarone on Cav3.1- and Cav3.2-T-type calcium channel current

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ABSTRACT

T-type calcium channels (TTCCs), including the Cav3.1 and Cav3.2 subtypes, play a critical role in regulating cardiac electrical activity. Their abnormal expression is closely associated with arrhythmias and cardiac dysfunction caused by myocardial infarction (MI). Dronedarone, a multi-target antiarrhythmic drug, is widely used to treat atrial fibrillation and atrial flutter but has shown adverse effects under pathological conditions such as MI. This study aims to investigate the direct effects and underlying mechanisms of dronedarone on TTCCs. Using MI rat model, neonatal rat cardiomyocytes, and HEK293T cells transfected with Cav3.1 and Cav3.2, we systematically analyzed the effects of dronedarone on TTCC activity. In vivo experiments demonstrated that dronedarone exacerbated myocardial damage and fibrosis in the MI model. Dronedarone significantly inhibited Cav3.2 channel activity with an IC₅₀ of 9.20 μ M, while its effect on Cav3.1 was weaker (IC₅₀ = 46.41 μ M). Chronic treatment with dronedarone also slowed the activation and inactivation kinetics of TTCCs. These findings suggest that dronedarone inhibits TTCC activity, particularly the Cav3.2 subtype, and alters its kinetic properties. This study reported the electrophysiological effects of dronedarone on T-type calcium channels, providing new insights into its electrophysiological properties and potential role in adverse cardiac events.

Key words: T-type calcium channel; myocardial infarction; dronedarone; CACNA1G; CACNA1H.

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Introduction

T-type calcium channels (TTCCs) are critical ion channels involved in the regulation of cardiac electrical activity and calcium homeostasis. They are widely distributed in the sinoatrial node, atrioventricular node, and, under pathological conditions, in ventricular cardiomyocytes.¹ TTCCs primarily consist of two subtypes: Cav3.1 (CACNA1G) and Cav3.2 (CACNA1H), playing essential roles in low-voltage-activated depolarization, trigger activity, and calcium influx. Recent studies have shown that abnormal expression of TTCCs is closely associated with arrhythmias and cardiac dysfunction in pathological conditions such as myocardial infarction (MI).²⁻⁴

Dronedaron is a multi-target antiarrhythmic drug widely used in the treatment of atrial fibrillation and atrial flutter.^{5,6} However, clinical studies and animal experiments have revealed that dronedaron may exert adverse effects on cardiac function under certain pathological conditions, such as its negative inotropic effects.⁷ Previous research has reported that dronedaron reduces the maximum upstroke velocity of action potentials and prolongs action potential duration,⁸ but its specific mechanisms of action remain unclear.⁹

This study aims to investigate the direct effects and underlying mechanisms of dronedaron on T-type calcium channels. Using both acute and chronic treatment models, we systematically examined the effects of dronedaron on Cav3.1 and Cav3.2 subtypes transfected in HEK293T cells, focusing on its impact on the kinetic properties of TTCCs after MI. These findings provide new insights into the electrophysiological effects of dronedaron and the molecular mechanisms underlying its potential to impair cardiac function.

Materials and Methods

Animals

Adult male Wistar rats (10-12 weeks old, body weight 250-300 g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). The rats were housed in the IVC system (22-24°C, relative humidity 50-60%), with a 12 h/12 h light/dark cycle, and *ad libitum* access to food and water. Randomly divide the rats into three groups: Control group, MI group, and MI+Drug group (administer dronedaron *via* gavage at 30 mg/kg/d, after MI). After eight weeks of feeding, Echocardiography and ECG were measured on the animals. All experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals, and were approved by the Animal Care and Ethics Committee of Hebei Medical University.

Animal model of MI: rats of 8-10 weeks of age were used to establish the MI animal model. The animals were fixed on a plate at a constant temperature of 37°C, and the precordial area was routinely sterilized with povidone-iodine. The rats were preoperatively anesthetized with 3% isoflurane (1 L/min) by inhalation. An opening of approximately 2 cm was cut in the chest at the location of the most intense heartbeat with sterile scissors. A 4-0 purse-string was made and the thoracic cavity directly above the heart was incised through the fourth intercostal space using curved forceps. Gently press on the chest to extrude the heart from the chest cavity and place the heart in the sutured opening. At this point, make sure there is as little air in the chest cavity as possible. A surgical live knot is tied over the left anterior descending coronary artery using a 6-0 surgical suture, with one end of the suture

exposed outside the chest cavity. The heart was immediately placed back into the thoracic cavity, the excess air was expelled, and the ribcage was sutured closed. After surgery, the mice were returned to the anesthesia room.

Echocardiography

Rats were anesthetized with isoflurane (induced with 2.5%, maintained with 1.5%), and secured to the operating table in a supine position. After shaving the chest hairs and venous cannulation procedure, the rats were examined by left ventricular long axis B-mode ultrasound with a Vevo 2100 Imaging System (Fujifilm VisualSonics, Toronto, Canada) with a high-frequency linear array transducer probe (MS250, Fujifilm VisualSonics).¹⁰ Three consecutive cardiac cycles were used to analysis heart rate (HR), ejection fraction (EF), fractional shortening (FS) stroke volume (SV), cardiac output (CO), *etc.* These data were analyzed by 3-group ANOVA and Tukey's *post-hoc* test.

ECG recording *in vitro*

Experimental animals were anesthetized and placed in the supine position on the operating table. Their limbs were gently secured using specialized holders to ensure stability during the procedure. The skin was carefully cleaned to remove any impurities or hair that might interfere with electrode contact. Lead electrodes were then attached to the designated positions, and electrocardiogram (ECG) recordings were obtained using the BF-420F system (Chengdu Taimeng Technology Co., Ltd., Chengdu, China). The data were recorded and saved for each animal. Heart rate (HR), rhythm, and waveform parameters were subsequently analyzed. Statistical comparisons between groups were performed using 3-group ANOVA and Tukey's *post-hoc* test.

Histopathological analysis

H&E staining process

Rat hearts were fixed in paraffin, sectioned, and deparaffinized in xylene (twice for 5 min each). The sections were then rehydrated through a graded ethanol series (100%, 95%, 85%, and 70%) and rinsed in distilled water. Hematoxylin staining was performed for 5-10 min to visualize the cell nuclei in blue-purple. The slides were rinsed with running water to remove excess stain and briefly differentiated in 1% hydrochloric acid ethanol for a few seconds to eliminate nonspecific staining. After thorough washing, the nuclei appeared clear and blue-purple. The sections were subsequently counterstained with eosin for 2-3 min to stain the cytoplasm and extracellular matrix pink, followed by a quick rinse to remove excess dye. Finally, the sections were dehydrated, cleared, and mounted. Under microscopic observation, the nuclei appeared blue-purple, while the cytoplasm and extracellular matrix were pink.

Masson trichrome staining process

Tissue processing was performed as previously described, including deparaffinization and rehydration. The sections were then stained with Weigert's iron hematoxylin solution for 5-10 min, rendering the cell nuclei blue-black, followed by rinsing with running water to remove excess stain. Next, the sections were stained with 1% aniline green solution for 10-15 min to color the muscle, cytoplasm, and keratin proteins. After rinsing to remove residual dye, the sections were treated with phosphotungstic acid solution for 5-10 min to ensure selective staining of collagen fibers. Subsequently, the sections were restained with aniline green solution for another 5-10 min, producing blue or green collagen fibers.

After a final rinse with distilled water, the sections were dehydrated, cleared, and mounted. Under microscopic examination, the cell nuclei appeared blue-black, collagen fibers were stained blue or green, and muscle, cytoplasm, and other components appeared red.

Immunohistochemical staining

After routine deparaffinization and rehydration as described for H&E staining, antigen retrieval was carried out in citrate buffer (pH 6.0). The sections were blocked with 5% bovine serum albumin and incubated with a CaV3.2 Monoclonal Antibody (N55/10), FITC (1:50, cat.#: MA5-45396; Thermo Fisher Scientific, Waltham, MA, USA) or CaV3.1 Monoclonal Antibody (N178A/9), PE (1:50, cat.#: MA5-45546; Thermo Fisher Scientific) overnight at 4°C. After washing with PBS, the sections were counterstained with DAPI and mounted with an antifade mounting medium.

Isolation of primary myocardial cells

Neonatal Wistar rats (1–3 days old) were taken, and their skin was disinfected. The heart was quickly excised and placed into PBS to wash off excess blood. The cardiac tissues were minced into small pieces and digested in 5 mL of collagenase solution (0.4 g/L; Roche, Basel, Switzerland) in a 37°C water bath with stirring 3 times, each lasting 7–8 min. The supernatant was collected, and 1 mL of FBS was added to stop the digestion. The supernatants were centrifuged at 1000 rpm for 5 min at room temperature, and the supernatant was discarded. The cells were resuspended in DMEM (Thermo Fisher Scientific) standard medium. After 1 h of differential adhesion, the supernatant was collected and centrifuged to isolate relatively pure cardiomyocytes. The cells were cultured for subsequent experiments to test the current between 24–48 h.

HEK 293T cells culture and transfection

HEK 293T cells were cultured in standard medium (DMEM supplemented with 10% FBS and 1% penicillin-streptomycin) at 37°C incubator with 5% CO₂. When cell density reaches 70–80% confluence, transfection use the GP-transfect-mate reagent according to the manufacturer's instructions, adding the mixture and plasmid (constructed by Yaohe Biomed, with the target gene: CACNA1G; GenBank: KJ535064.1, and CACNA1H; GenBank: AF051946.3 use pLVX-puro fused to a C-terminal 3*Flag tag). The cells were then incubated for an additional 24 h to allow for Cav3.1 and Cav3.2 expression, after which the patch clamp was carried out as planned.

Patch clamp recordings in HEK cells and primary myocardial cells

EPC-10 amplifier (HEKA Elektronik, Lambrecht, Germany) was used to record current of T-type calcium channel. Acquisition and analysis were performed using Patchmaster software (Pulse/Pulsefit v.8.6, HEKA Elektronik). Glass capillaries (Sutter, Instrument Co., Novato, CA, USA) pulled with a micropipette puller, Model P-97 (Sutter Instrument Co.), are filled with electrode solution to achieve a tip resistance of 3–5 MΩ. The recording chamber was filled with the bath solution of the following components (mmol/l): tetraethylammonium chloride, 120; CsCl, 6; 4-aminopyridine, 5; MgCl₂, 0.5; 4,4-diisothiocyanato stilbene-2,2-disulfonic acid, 0.1; HEPES, 10; CaCl₂, 1.8; glucose, 10 and tetrodotoxin (TTX), 0.01 (the pH was adjusted to 7.4 with 1 N TEA-OH), where TTX was omitted in studies of the recombinant system. The patch-clamp electrode was filled with the pipette solution of (mmol/l): CsCl, 130; MgCl₂, 2; ATP, 2; GTP, 0.5; EGTA, 5

and HEPES, 5 (the pH was adjusted to 7.3 with 1 N CsOH). The voltage stimulation protocol was based on previously published literature.¹¹ Different holding potentials of -100 or -50 mV was used to identify I_{Ca,L} and I_{Ca,T}. That is because at -100 mV, both T-type and L-type channels are available, while at -50 mV, T-type channels are largely inactivated, leaving only L-type currents. You will get the T-type calcium current by subtracting the L-type channel current at each voltage.

HEK 293T cells transfected 24–48 h using 1.5 µg of the CACNA1G or CACNA1H gene or the single cells isolated from 1- to 3-day-old Wistar rats are used for patch clamp. Single cells with a regular shape are selected to record. Dronedarone was used by increasing concentrations of 0.1, 0.3, 1, 3, 10, and 30 µM in acute effection experiment. To analyze the function we need to fit the IC50 curves and the activation and inactivation curve. Additionally, we use 1 µM (cells will dead if higher concentration) incubate cells 48 h for chronic experiment and compared the electrophysiological properties before and after incubation.

Dose-response relationship on I_{Ca,T} fitted by the Hill equation: $(Y = Y_{max} * (1 / (1 + (K_{1/2}/x)^n)))$ (GraphPad Prism8.2.1). I-V curve was fitted by Nonlinear fitting. Student's t test was used for bar graph to compare the current before and after dronedarone application in -20 mV (SigmaPlot SPW12.0). Activation curve fitted with Boltzmann equation: $G/G_{max} = [1 + \exp((V_{1/2-act} - V)/k)]^{-1}$, where G_{max} is the maximum conductance, V_{1/2-act} is the half-activation potential and k is the slope factor. The steady-state inactivation curve was fitted with an equation: $I/I_{max} = [1 + \exp((V - V_{1/2-ss})/k)]^{-1}$, where V_{1/2-ss} is the half-inactivation potential. current-voltage (I-V) relationship was fitted use a Boltzmann distribution function: $I = G_{max} (V_m - V_{rev}) / (1 + \exp((V_m - V_{1/2})/k))$. I is the peak of I_{Ca,T} at the test potential V_m, V_{rev} is the reversal potential, G_{max} is the maximal slope conductance, V_{1/2} is the half-point of the relationship and k is the slope factor (OriginPro 2016).

Western blotting

HEK293T cells transfected with CACNA1H were treated with dronedarone under normal and ischemic-hypoxic (1% O₂ concentration in incubator) conditions for 48 h followed by Western blot (WB) analysis. Cells were lysed with lysis buffer, and proteins were extracted by centrifugation. Electrophoresis was performed at 80 V for 2 h, followed by membrane transfer at 60 V for 2.5 h. The membrane was blocked with BSA for 30 min. The primary antibody used was DYKDDDDK Tag (FLAG) Antibody (dilution 1:5000, HY-P80111; MedChemExpress, Shanghai, China), and the secondary antibody was Anti-mouse IgG(H+L) (dilution 1:10000, Cat: 33201ES60; Yeasen, Shanghai, China). After antibody washes, chemiluminescence detection was performed. The FLAG-tagged protein was detected using a luminometer (Odyssey Clx, LI-COR Biosciences, Lincoln, NE, USA), and the results were analyzed with ImageJ software.

Statistical analysis

Statistical analysis was performed using GraphPad Prism version 8 (GraphPad Software Inc., La Jolla, CA, USA) and OriginPro software (OriginLab, Northampton, MA, USA). Data was done the normality test before ANOVA and I-test. All data were expressed as mean ± SEM. A p-value < 0.05 was statistically significant.

Results

Dronedarone does not improve impaired cardiac function after myocardial infarction

To investigate the effects of dronedarone on cardiac function in rats with myocardial infarction, we divided the rats into three groups: control (NC), myocardial infarction (MI), and MI +

dronedarone-treated groups. Rats were gavage-fed 14 d with vehicle or dronedarone (30 mg/kg/d), after 8 weeks of MI,¹² conducted assessments using echocardiography, electrocardiograms (ECG), and histological analyses. The results showed that dronedarone did not improve cardiac systolic function (Figure 1 A-C) or the prolonged QRS and QT intervals (Figure 1 D-F) after myocardial infarction. Histological examinations with H&E and Masson staining further revealed that dronedarone exacerbated myocardial morphological damage and increased fibrotic infiltration (Figure 1

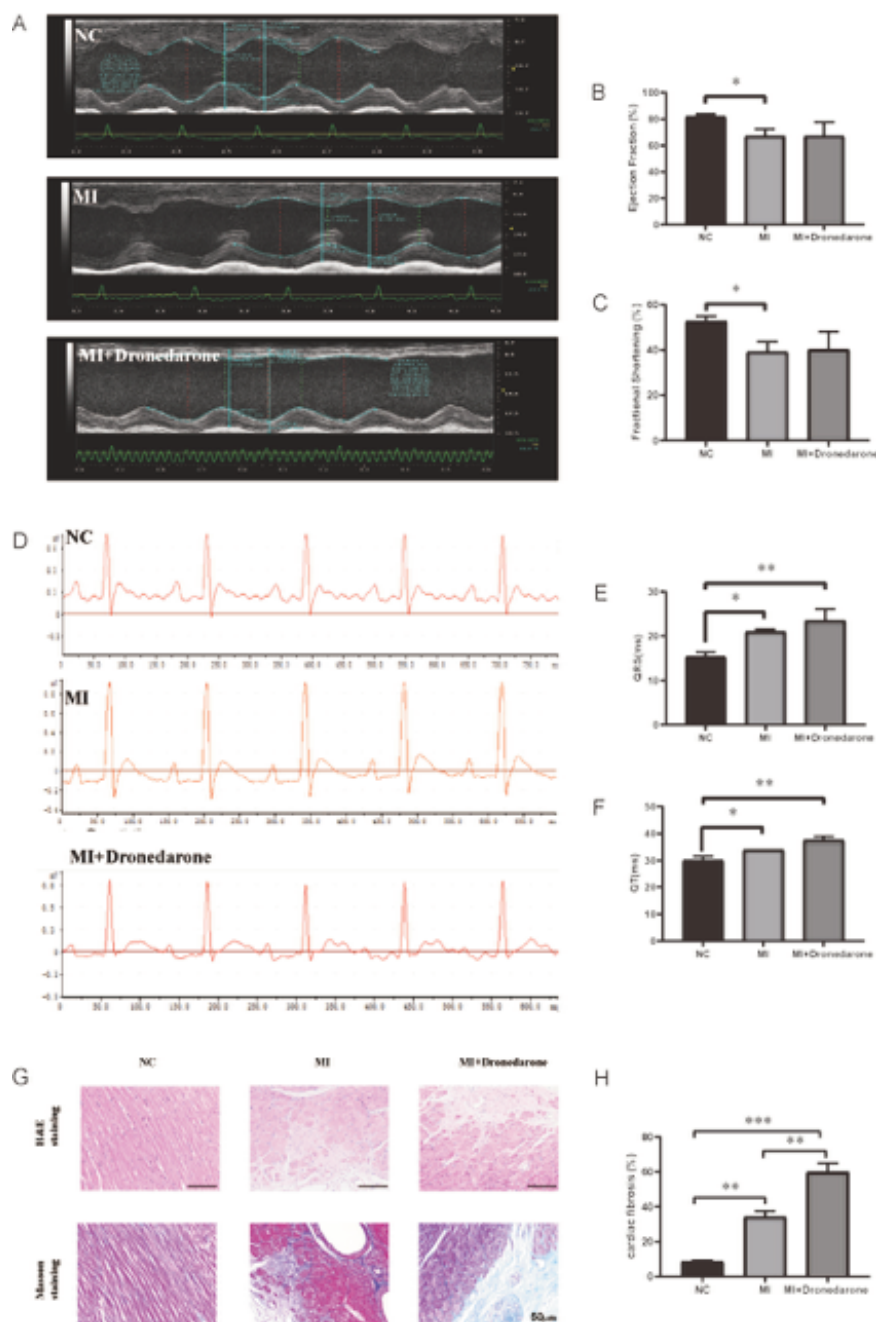


Figure 1. Dronedarone do not improve cardiac function in myocardial infarction (MI) rats. **A)** Representative echocardiographic images of control, MI, and MI + dronedarone groups. **B,C)** Statistical plots of ejection fraction (EF) and fractional shortening (FS). **D)** Representative electrocardiographic images of Control, MI, and MI + dronedarone groups. **E,F)** Statistical plots of QRS interval and QT interval. **G, H)** Representative histological images and statistical plots of heart tissue sections from control, MI, and MI + dronedarone groups. n=4-5; * $p < 0.05$, ** $p < 0.01$.

G,H). In summary, dronedarone failed to improve myocardial tissue morphology or function and even worsened the condition.

Re-expression of T-type calcium channel after myocardial infarction

Since dronedarone causes severe damage in MI models, what could be its underlying mechanism? It is known from previous studies that dronedarone (at concentrations of 3, 10, and 30 μM) can reduce the maximal upstroke velocity of action potentials (AP) and prolong the action potential duration (APD) in normal hearts.⁸ We hypothesize that dronedarone may affect the activation or inactivation properties of T-type calcium channels. To explore this, we examined the expression of Cav3.1 and Cav3.2 in three groups of rats and found that T-type calcium channels expression were decreased after myocardial infarction with dronedarone (cav3.2 expression from 23.00 ± 1.52 to 17.00 ± 1.15 , $p < 0.05$, Figure 2 A-C). Further experiments using HEK293T cells transfected with Cav3.2 and also confirmed a significant upregulation of Cav3.2 expression under normoxic or ischemic-hypoxic conditions (1% O_2) for 48 h. (from -0.58 ± 0.13 to 1.10 ± 0.05 , $p < 0.01$, Figure 2 D,E). Based on these findings, we propose that expression of T-type calcium channels, which could contribute to its observed detrimental effects in MI models.

Dronedarone inhibits T-type calcium channel activity in neonatal rat cardiomyocytes

To verify whether dronedarone affects T-type calcium channels in cardiomyocytes, we used neonatal rat cardiomyocytes (2-3 days old) and measured the total $I_{\text{Ca,L+T}}$ current and $I_{\text{Ca,L}}$ current separately. The results demonstrated that dronedarone significantly inhibited both L-type and T-type calcium channels (Figure 3A). Curve fitting of the inhibition percentage of T-type calcium channels at different dronedarone concentrations indicated an IC_{50} value of 3.682 μM (Figure 3B). Figure 3C presents the I-V curve at 5 μM , and analysis of the current density of T-type calcium channels at -30 mV (peak) revealed a significant difference (from -4.18 ± 0.58 pA/pF to -1.71 ± 0.36 pA/pF, $p < 0.01$, Figure 3D).

Dronedarone inhibits $I_{\text{Ca,T}}$ ($\alpha 1\text{H}$) channel activity

To further investigate whether dronedarone exerts its effect on T-type calcium channels through the Cav3.1 or Cav3.2 subtype, we transfected human CACNA1G (Cav3.1) and CACNA1H (Cav3.2) into HEK293T cells. Figure 4 shows the response of the HEK-293/ $\alpha 1\text{H}$ (Cav3.2) subtype to dronedarone. The results indicate an IC_{50} of 9.20 μM (Figure 4B). At a concentration of 10 μM dronedarone, the I-V curve (Figure 4C) and current density at -40 mV (peak) were significantly inhibited (from -5.64 ± 0.85 pA/pF to -1.59 ± 0.40 pA/pF, $p < 0.01$, Figure 4D). Additionally, both the acti-

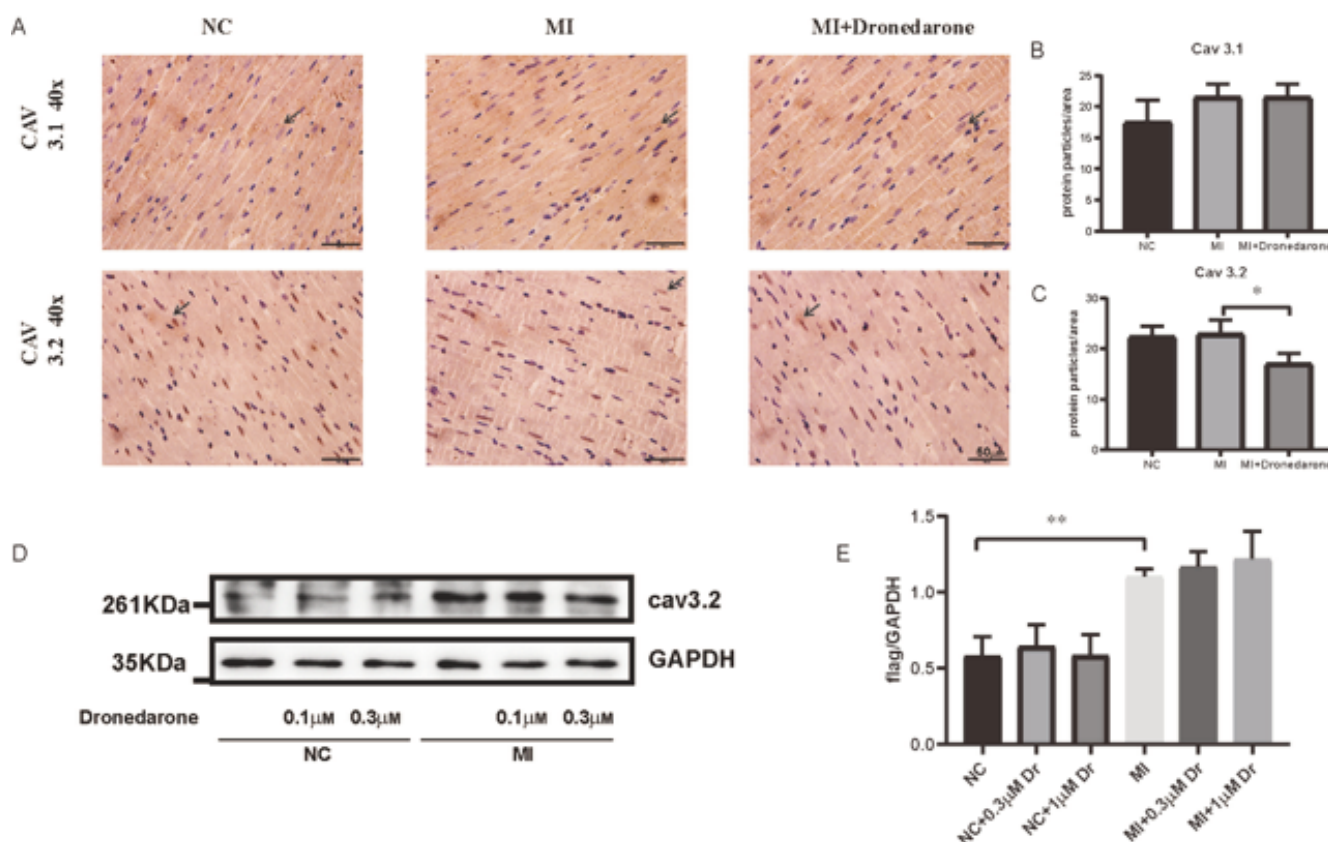


Figure 2. Dronedarone improved the expression of T-type calcium channels in myocardial infarction (MI) rats. Representative immunohistochemical staining (A) and analysis (B,C) of Cav3.1 and Cav3.2 in control, MI, and MI + dronedarone groups. Western blot images (D) and analysis (E) of HEK293 cells transfected with Cav3.2. ** $p < 0.01$.

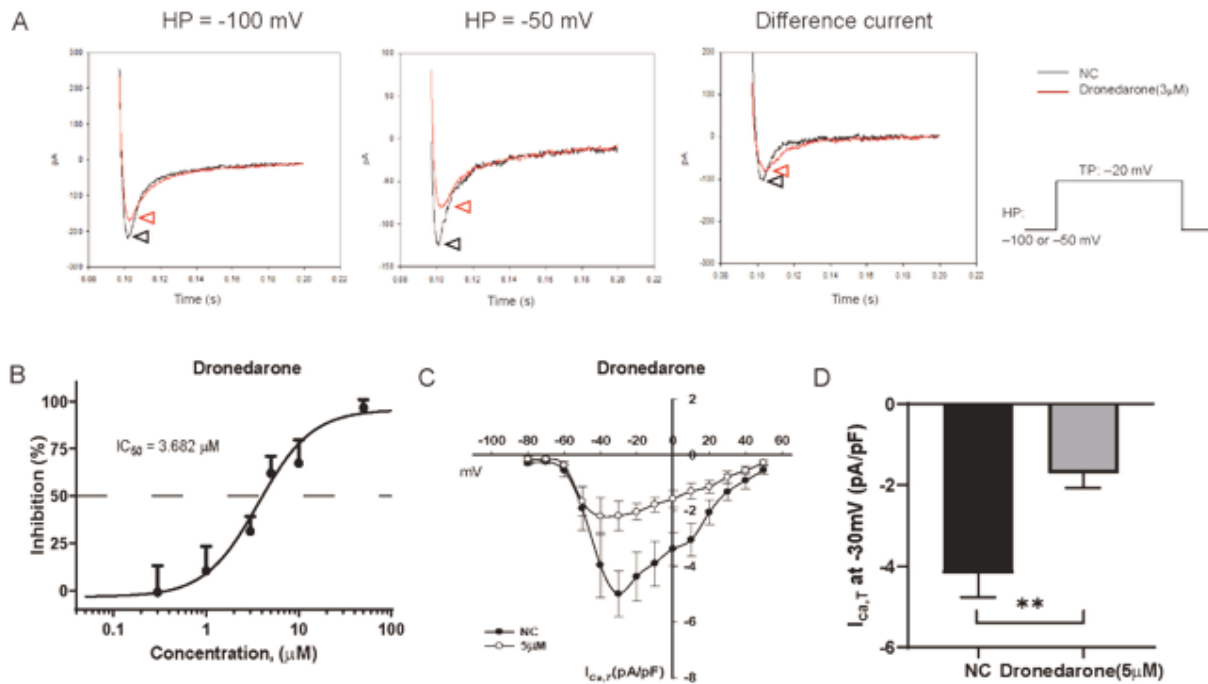


Figure 3. Dronedarone inhibited T-type calcium channel activity in neonatal rat cardiomyocytes. **A)** Representative current traces showing the effects of dronedarone before (black) and after application (red) on L+T, L-, and T-type calcium channel currents. **B)** Inhibition curve of T-type calcium channel currents by dronedarone. **C)** I-V curve analysis of T-type calcium channels before (solid circles) and after dronedarone application (open circles). **D)** Statistical comparison of T-type calcium channel current density before and after dronedarone application. N, number of rats ($n=5$); n, number of cells ($n=5-10$); $**p<0.01$.

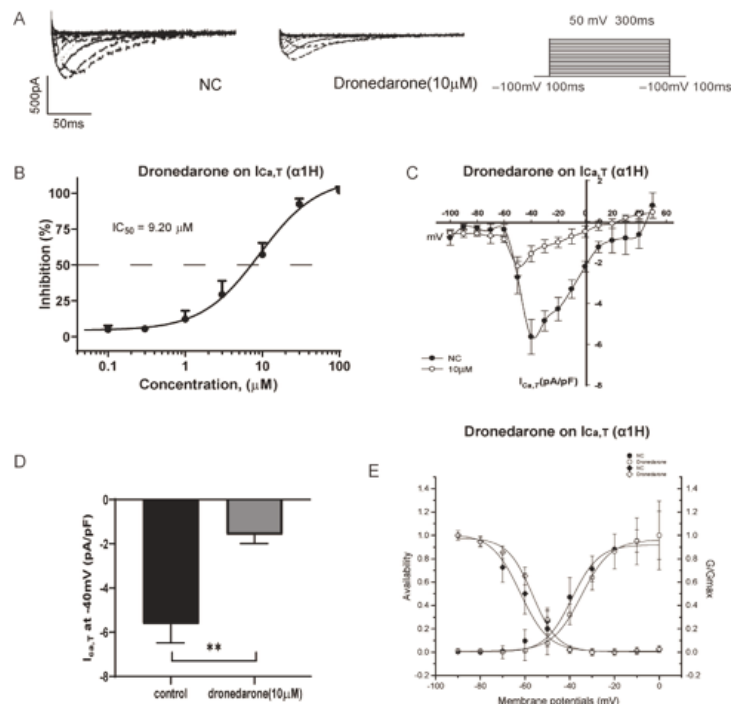


Figure 4. Dronedarone acutely inhibits the activity of the Cav3.2 subtype T-type calcium channel. **A)** Representative current traces showing the effects of dronedarone before and after application on Cav3.2 channel currents. **B)** Inhibition curve of Cav3.2 channel currents by dronedarone. **C)** I-V curve analysis of Cav3.2 channels before (solid circles) and after dronedarone application (open circles). **D)** Statistical comparison of Cav3.2 channel current density before (black) and after (grey) dronedarone application. **E)** Activation (G/G_{max}) and the steady-state inactivation (availability) curves of Cav3.2 channels before and after dronedarone application. Number of cells: 4-5; $**p<0.01$.

vation and inactivation curves have a right shift (4.25 mV and 5.56 mV), indicating altered channel kinetics (Figure 4E). Similarly, the effect of dronedarone on the Cav3.1 subtype was examined (Figure 5A) and found to be less pronounced than its effect on Cav3.2, with an IC_{50} of 46.41 μ M (Figure 5B). At a concentration of 40 μ M dronedarone, the I-V curve and current density at -40 mV showed a trend of inhibition (Figure 5 C-E).

Chronic treatment with dronedarone also alters the properties of T-type calcium channels

Additionally, considering that *in vivo* models involve chronic processes, we pretreated HEK293 cells transfected CACNA1H (Cav3.2) with dronedarone for 48 h before testing (Figure 6A). The results showed similar findings to the acute response. There is a right shift of about 5 mV in activation curve ($V_{1/2-act}$: -43.1 mV in control vs -38.0 mV with dronedarone) and 8 mV in the steady-state inactivation curve ($V_{1/2-SSI}$: -60.8 mV in control vs -52.5 mV with dronedarone). Slope factors (k) decreased from 7.2 mV to 4.8 mV in activation curve and from 9.0 mV to 4.3 mV in inactivation curve (Figure 6 B,C).

Discussion

Dronedarone, a non-iodinated benzofuran derivative, is widely used for the treatment of atrial fibrillation (AF) and atrial flutter (AFL) due to its multi-channel blocking properties.^{13,14} As an analogue of the classic amiodarone, it has been extensively applied in both clinical and animal studies.¹⁵⁻¹⁹ Dronedarone inhibits multiple

channels, including transmembrane Na^+ , K^+ , Ca^{2+} , and slow L-type calcium currents.⁶ However, its effects on T-type calcium channels (TTCCs) have not been reported. Thus, we defined our research objective to explore the effects of dronedarone on TTCCs and its impact on cardiac electrical activity, evaluating its potential therapeutic implications. The inhibitory effect of dronedarone on T-type calcium channels, especially Cav3.2 subtype.

Although T-type calcium channels are predominantly expressed in sinoatrial node cells, their re-expression has been reported in ventricular myocardium under pathological conditions such as cardiac hypertrophy and heart failure.¹ We focused on ventricular tissue to investigate the potential role of T-type calcium channel in ventricular remodeling. In our myocardial infarction (MI) rat model treated with dronedarone, we observed a prolonged QT interval.²⁰ Previous studies have reported that dronedarone reduces the action potential (AP) rate.¹³ Therefore, we hypothesized that TTCCs, in addition to the classical L-type calcium channels, might also contribute to this process. Using neonatal rat cardiomyocytes, we tested our hypothesis and confirmed that dronedarone inhibits TTCCs with an IC_{50} of 3.682 μ M. Also in HEK293T cells transfected with CACNA1G and CACNA1H showed dronedarone significantly shifts the T-type window current towards more positive potentials despite the reduction in current density. The more depolarized potentials may increase the probability of channel opening within a physiologically voltage. Such persistent inward Ca^{2+} current could delay polarization and thereby contribute to QT interval prolongation.

Next, we investigated the acute effects of dronedarone on the Cav3.1 and Cav3.2 subtypes. We found that dronedarone signifi-

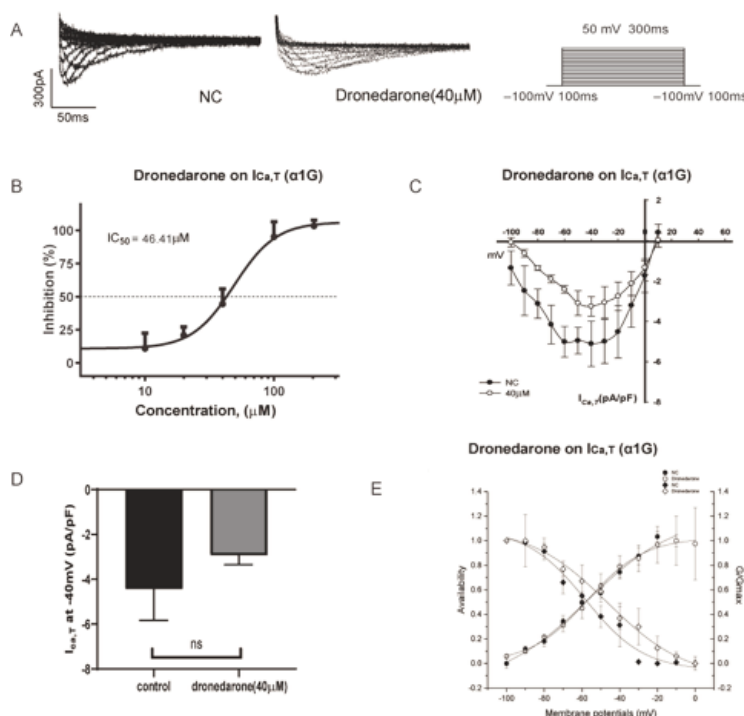


Figure 5. Short-term effects of dronedarone on Cav3.1 T-type calcium channels. **A)** Representative current traces showing the effects with or without dronedarone on Cav3.1 channel currents. **B)** Dose-response curve of Cav3.1 channel currents with dronedarone. **C)** I-V curve analysis of Cav3.1 channels before (solid circles) and after dronedarone application (open circles). **D)** Statistical comparison of Cav3.1 channel current density before (black) and after (grey) dronedarone application. **E)** Activation and steady-state inactivation curves of Cav3.1 channels before (solid) and after (open) dronedarone application. Number of cells: 3-5.

cantly inhibited Cav3.2 channel activity, with an IC_{50} of 9.20 μ M, while its inhibition of Cav3.1 channels was relatively weaker (IC_{50} =46.41 μ M). This discrepancy might be attributed to structural differences between channel subtypes affecting drug sensitivity or changes in oxidative stress levels induced by dronedarone.

Under chronic conditions, due to the prolonged culture and modeling time constraints in neonatal cardiomyocytes, we opted to use transfected cells for this part of the study to replicate the effects observed in animal models. After 48 h of culture, we selected a drug concentration of 1 μ M based on preliminary testing and found that dronedarone also inhibited TTCC activity. However, in animal models, we must consider that dronedarone acts as a multi-channel inhibitor, with potential contributions from Na^+ and K^+ channels.²¹ Also pro-inflammatory effects is not excluded.

Furthermore, in the whole animal model, we observed that dronedarone might exacerbate myocardial fibrosis after MI. Fibrotic markers, including α -SMA, OPN, COL1, and COL3, showed an increasing trend. Based on previous clinical reports on fibrosis, this effect might be associated with oxidative stress responses in the vasculature induced by dronedarone.^{5,14} The specific mechanisms require further investigation with Cav3.2-specific inhibition/knockdown model to confirm the causal link.

In summary, our study has some limitations: Firstly, using (-100) mV and (-50) mV holding potentials and subtracting currents may not fully isolate L- and T- type calcium channel in neonatal cardiomyocytes. Secondly, our research is primarily based on neonatal rat cardiomyocytes and transfected HEK293T cells, without further validation in adult cardiomyocytes. Thirdly, we have not thoroughly investigated the specific molecular mechanisms underlying the inhibition of T-type calcium channels by dronedarone, such as whether it directly binds to channel proteins or indirectly regulates them through signaling pathways. Based on the limitations, we need to consider the blockers (TTA-P2 for T-type channels or nifedipine for L-type channels) in neonatal car-

diomyocytes experiments. The purpose of next research focus will be to explore the precise mechanisms by which dronedarone affects T-type calcium channels. We plan to use molecular docking simulations such as R1548P,²² S196L, M1549I, V1951E and P2083L,²² and site-directed mutagenesis experiments to determine whether dronedarone directly binds to specific sites on Cav3.2, thereby inhibiting its activity. Additionally, we will investigate whether modulating protein kinases, such as PKA²⁴ and CaMKII,²⁵ or oxidative stress-related signaling pathways²⁶ participate in the regulation of T-type calcium channel expression and function with dronedarone.

Overall, our study suggests that T-type calcium channels may serve as an important target of dronedarone in post-myocardial infarction conditions, offering new insights into its electrophysiological effects. Future research should focus on the long-term effects of dronedarone under different pathological conditions, utilizing gene-editing technologies to explore its specific regulation of T-type calcium channels. It may provide novel strategies for the precise treatment of arrhythmias and myocardial injury.

In summary, our study demonstrates that dronedarone significantly inhibits T-type calcium channels in cardiomyocytes, with distinct effects on the Cav3.1 and Cav3.2 subtypes. Acute treatment showed a stronger inhibitory effect on Cav3.2, with an IC_{50} of 9.20 μ M, compared to Cav3.1, with an IC_{50} of 46.41 μ M. Also, dronedarone suppressed current density and altered channel activation and inactivation properties. Chronic treatment with dronedarone, mimicking *in vivo* conditions, produced similar effects. These changes suggest that dronedarone modulates T-type calcium channel kinetics, potentially contributing to its deleterious effects on myocardial function in post-myocardial infarction models and provide new insights into its cardiac electrophysiological effects. This is the first direct report on the electrophysiological effects of dronedarone on T-type calcium channels.

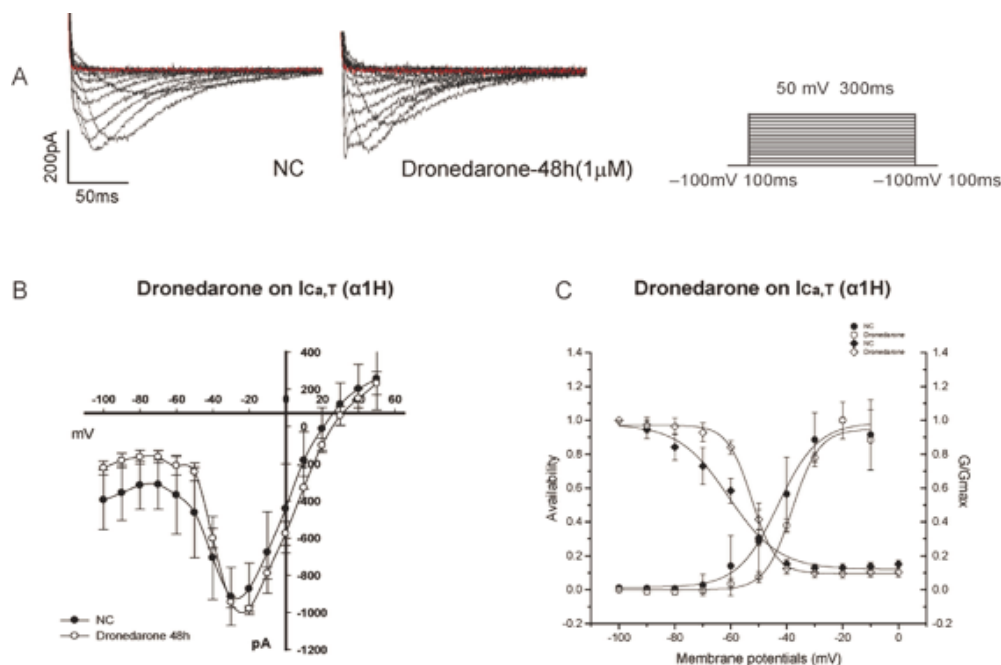


Figure 6. Chronic effects of dronedarone on Cav3.2 T-type calcium channels. **A)** Representative current traces showing the effects of dronedarone application (48 h) on Cav3.2 channel currents. **B)** I-V curve analysis of Cav3.2 channels before (solid circles) and after (open circles) 48 h dronedarone application. **C)** Activation and steady-state inactivation curves of Cav3.2 channels before (solid circles) and after (open circles) 48 h dronedarone application. Number of cells: 8-10.

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