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Effects of Zacopride, a Moderate I_{K1} channel agonist, on Triggered Arrhythmia and Contractility in Human Ventricular Myocardium

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Abstract

Ventricular tachycardia is the leading cause of sudden arrhythmic death in the U.S. Recently, the moderate I_{K1} channel activator, zacopride, was shown to suppress triggered ventricular tachycardia in rats. Nonetheless, concerns were raised about the possibility of pro-arrhythmic activity after I_{K1} channel stimulation based on the promising anti-arrhythmic strategy of I_{K1} blockade in other animal models. Therefore, the goal of the current study was to investigate the *ex-vivo* effects of zacopride on triggered arrhythmia and contractility in ventricular human myocardium in order to validate data that was solely obtained from animal models. Application of 100 nmol/L isoproterenol and 0.5 mmol/L caffeine led to triggered arrhythmia in isolated cardiac muscles from non-failing and end-stage failing hearts. However, the occurrence of arrhythmia in muscles of non-failing hearts was markedly higher than those of end-stage failing hearts. Interestingly, zacopride eliminated the *ex-vivo* triggered arrhythmia in these muscles of non-failing and failing hearts in a concentration-dependent manner, with an effective IC_{50} in the range of 28 to 40 μ mol/L. Conversely, in the absence of isoproterenol/caffeine, zacopride led to a negative inotropic effect in a concentration-dependent manner. Reduced cardiac contraction was clearly observed at high

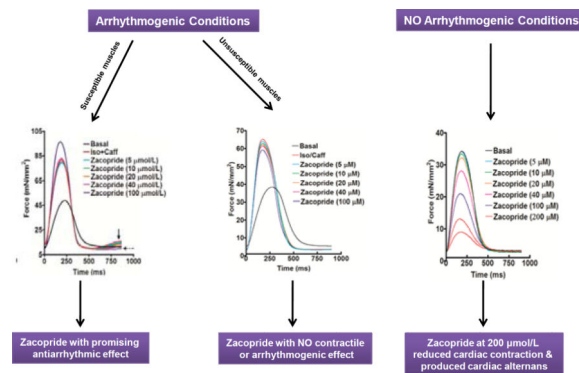
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Conflicts-of-interest
None.

zacopride concentration of 200 $\mu\text{mol/L}$, along with the occurrence of contractile alternans in muscles of non-failing and failing hearts. Zacopride shows promising antiarrhythmic effects against triggered arrhythmia in ventricular human myocardium. However, in the absence of Ca^{2+} overload/arrhythmia, zacopride, albeit at high concentrations, decreases the force of contraction and increases the likelihood of occurrence of contractile alternans, which may predispose the heart to contractile dysfunction and/or arrhythmia. Overall, our results represent a key step in translating this drug from the benchtop to the bedside in the research area.

Graphical Abstract



Keywords

Zacopride; $\text{I}_{\text{K}1}$ channel; Triggered Arrhythmia; Contractility; Human Heart

Introduction

Ventricular tachycardia (VT) due to irregular ectopic beats is the leading cause of sudden arrhythmic death in the U.S [1, 2]. VT occurs in patients with structural heart disease [3] as well as in apparently healthy individuals mostly during exercise or stress-induced catecholamine flow [4]. Under different settings, abnormal diastolic Ca^{2+} release (DCR) through the ryanodine receptor (RyR2) on the sarcoplasmic reticulum (SR) causes this ectopic activity mainly in the form of delayed after depolarizations (DADs), resulting in triggered arrhythmias [5–8].

The cardiac inward rectifier potassium channel ($\text{I}_{\text{K}1}$) is considered as the major conductance entity that regulates the resting potential (RP) and allows a considerable repolarization current during the last action potential (AP) phase. This big milieu of $\text{I}_{\text{K}1}$ conductance generates very little resting membrane resistance. Yet, when the $\text{I}_{\text{K}1}$ current reduction augments membrane resistance, a specified depolarizing membrane current could lead to a great extent of voltage deflection. This finding could signify a key mechanism for the occurrence of DADs-induced arrhythmias [9]. In cardiomyocytes from failing rat hearts, enhanced Ca^{2+} release through RyR2 has been reported to inhibit $\text{I}_{\text{K}1}$, signifying a substrate for VT in heart failure (HF) [10]. Interestingly, a rise in non-adrenergic $[\text{Ca}^{2+}]_i$ causes an increase of the $\text{I}_{\text{K}1}$ current in dogs and humans, regardless of the mechanism and may provide an effective endogenous defense against cardiac arrhythmias induced by Ca^{2+}

overload [11]. Also, during adrenergic stimulation, discrete modulatory pathways (e.g. PKA, PKC & CaMKII), through parallel I_{K1} targeting, may adjust it together and then develop the AP duration (APD) adaptation to varying conditions. Whereas adrenergic-induced activation of PKA and PKC reduces I_{K1} in both animals [12] and humans [13] and likely resulting in arrhythmia due to subsequently reduced repolarization reserve, the rise in $[Ca^{2+}]_i$ and following activation of CaMKII leading to improved I_{K1} may neutralize these effects and prevent cardiac arrhythmias [11]. Hence, an anti-arrhythmic approach should augment the I_{K1} to get it back to a near-normal condition. As previously noted, an ideal agent for the treatment of VT may be a drug that enhances the opening of K^+ channels at the RP but not at the AP peak, which means a drug that augments the I_{K1} current activation [9, 14]. However, the lack of I_{K1} -specific pharmacological tools, in particular a specific agonist, was a major constraint to further investigate I_{K1} in the past [14].

Recently, a novel discovery of I_{K1} channel agonist, zacopride, has been published [15]. Zacopride was originally reported as a potent antagonist of 5-hydroxytryptamine (5-HT)₃ and an agonist of 5-HT₄ receptors that has been experimentally used as an anti-emetic, gastroprokinetic, and anxiolytic agent [9]. Zacopride has been reported to selectively activate the I_{K1} channel in rat ventricular myocytes, moderately enhance the I_{K1} current, hyperpolarize the RP, and decrease the APD exclusive of affecting other channels, transporters, and pumps, with subsequent suppression of triggered VT in rats [15]. Conversely, data from other studies on primates, rabbits and rats, have shown I_{K1} blockade as a promising anti-arrhythmic strategy [16, 17], and thus concerns were raised about the possibility of pro-arrhythmic activity after I_{K1} channel stimulation [17]. Indeed, humans exhibit lower repolarization reserve contributions from I_{K1} and I_{Ks} compared to animals, confirming species-specific determinants of repolarization and limitations of animal models for human diseases [18]. Consequently, it has been concluded that the effects of the I_{K1} channel agonists on heart rhythm/arrhythmias may potentially be coupled with the type of arrhythmia and species, with the need for further future investigation in humans [9, 15].

In addition to their beneficial antiarrhythmic effects, several antiarrhythmic drugs have considerable influences on myocardial contractility. Mainly, they exert negative inotropic effects that restrict their clinical use in patients with compromised cardiac function [19]. Thus, investigating the impact of antiarrhythmic drugs on the contractile profile of cardiac muscle represents an important complementary safety measure to their therapeutic efficacy. This would provide more comprehensive knowledge not only about the clinical efficacy but also about possible side effects of these drugs on the heart, which is critical for their transition towards clinical application.

Therefore, the goal of the current study was to investigate the *ex-vivo* effects of the moderate I_{K1} channel activator, zacopride, on triggered arrhythmia and contractility in ventricular myocardium of non-failing and end-stage failing human hearts.

Methods

Human Tissue Collection

All human tissues were used with approval from the Institutional Review Board (IRB) of The Ohio State University and in accordance with the Declaration of Helsinki. Informed consents were acquired from cardiac transplant patients. Non-transplantable donor hearts were acquired from Lifeline of Ohio Organ Procurement. As described previously [20], explanted hearts were obtained in the operating room, flushed immediately after removal from living donors, and thereafter transferred to the laboratory in cold cardioplegic solution containing (in mM): 110 NaCl, 16 KCl, 16 MgCl₂, 10 NaHCO₃, and 0.5 CaCl₂. Ejection fraction of non-failing hearts, measured prior to procurement, was in the normal range with few exceptions. These exceptions did not translate to different results compared to other hearts with normal ejection fraction. None of the non-transplantable hearts had signs of coronary bypass surgery or prior myocardial infarctions. Wall thicknesses, heart weights, and heart weight-to-body weight ratios of these hearts were all in the normal range. Because it is not feasible to attain completely healthy “control” human hearts devoid of any sort of cardiovascular disease for research purposes since such hearts are almost exclusively utilized for cardiac transplantation, we opted to use the term “non-failing” as opposed to “control” in order to reflect this property as we described before [20]. End-stage failing hearts were acquired from patients undergoing cardiac transplantation at The Ohio State University Wexner Medical Center. All hearts, regardless of source, were processed with the identical protocol.

Trabeculae Isolation

Hearts were rapidly transferred from the cardioplegic solution to a cold modified Krebs–Henseleit buffer containing (in mmol/L): 120 NaCl, 5 KCl, 2 MgSO₄, 1.2 NaH₂PO₄, 20 NaHCO₃, 0.25 Ca²⁺, and 10 glucose, equilibrated with 95% O₂-5% CO₂, resulting in a pH of 7.4. Additionally, 20 mmol/L 2,3-butanedione monoxime (BDM) was added to the dissection buffer to prevent cutting injury [20]. Uniform linear trabeculae were carefully dissected from both right ventricle (RV) and left ventricle (LV), and then kept in this solution at 0–4°C until the time of the experiment (0–8 h). The dimensions of muscles were measured using a calibration reticule in the ocular of the dissection microscope (40×, resolution ~10 μm). The cross-sectional areas were calculated assuming ellipsoid cross-sectional shapes. Average dimensions (width × thickness × length) of RV and LV trabeculae from non-failing hearts were (0.33 ± 0.04 × 0.22 ± 0.03 × 3.03 ± 0.53 mm) and (0.34 ± 0.06 × 0.23 ± 0.04 × 3.16 ± 0.89 mm), respectively. Likewise, average dimensions of RV and LV trabeculae from end-stage failing hearts were (0.37 ± 0.04 × 0.25 ± 0.03 × 3.03 ± 0.47 mm) and (0.44 ± 0.05 × 0.30 ± 0.03 × 2.62 ± 0.22 mm), respectively.

With the use of the dissection microscope, muscles were mounted between the basket-shaped extension of a force transducer (KG7, Scientific Instruments, Heidelberg, Germany) and a hook connected to a micromanipulator as previously described [20, 21]. Muscles were superfused with the same buffer at 37°C as above (with the exception that BDM was omitted) and stimulated at close to physiologic frequency of 1 Hz. Extracellular Ca²⁺ concentration was raised to 2 mmol/L and muscles were allowed to stabilize for at least 30

minutes before the experimental protocol was initiated. Generally, muscles were stretched to an optimal length where a small increase in length resulted in nearly equal increases in resting tension and active developed tension. This length was selected to be comparable to the maximally attained length *in-vivo* at the end of diastole [22].

Arrhythmia Experiments

A total of 10 RV and 8 LV trabeculae from 10 different non-failing hearts, and 12 RV and 12 LV trabeculae from 14 different end-stage failing hearts were used in arrhythmia experiments. The characteristics of these hearts are provided in Table 1 and Table 2. These intact, twitch contracting trabeculae were allowed to develop a contractile homeostasis under near physiologic conditions (37 °C, 1 Hz at optimal length) in oxygenated Krebs-Henseleit buffer containing 2 mM Ca²⁺, and twitch amplitude and twitch kinetics were assessed at basal level. Once this data was collected, 100 nmol/L isoproterenol and 0.5 mmol/L caffeine were added to the perfusion solution to induce arrhythmia. Twitch contractions were monitored for 10 minutes for the development of any triggered activity. Zacopride was added at a concentration range of 5–100 µmol/L at 2 minute intervals, starting 1-to-3 minutes post arrhythmia; twitch data as well as triggered activities were recorded. Interval time and drug addition starting time were determined based on preliminary data showing that early addition of the drug is required for its antiarrhythmic efficacy (Supplementary Figure 1).

Contractility Experiments

In another group of muscles, we studied the direct effects of zacopride on the contractile profile of ventricular myocardium. A total of 5 RV and 4 LV trabeculae from 5 different non-failing hearts, and 7 RV and 6 LV trabeculae from 6 different end-stage failing hearts were used in contractility experiments. The characteristics of these hearts are provided in Table 3 and Table 4. These intact, twitch contracting trabeculae were allowed to develop a contractile homeostasis at near physiologic conditions (37 °C, 1 Hz at optimal length) in oxygenated Krebs-Henseleit buffer containing 2 mM Ca²⁺; twitch amplitude and twitch kinetics were assessed at basal level. Once this data was collected, zacopride (5–200 µmol/L) was added at 3 minute intervals and twitch amplitude and twitch kinetics were assessed again.

In all experiments, peak isometric developed force (F_{dev}) was determined and normalized to the cross-sectional area of the muscle. Additionally, as a force-independent parameter of force decay kinetics, time to peak force (TTP) and time from peak force to 50% relaxation (RT50) were determined.

Data Analysis and Statistics

Data are presented as mean ± SEM and were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett Multiple Comparisons post-hoc test, comparing all groups to isoproterenol/caffeine group in arrhythmia experiments and to basal control group in contractility experiments. A two-tailed value of P < 0.05 was considered statistically significant.

Results

At near physiologic conditions (37 °C, 1 Hz, 2 mM Ca²⁺ at optimal length), our data showed that muscles from non-failing hearts were more susceptible to isoproterenol/caffeine - induced arrhythmia compared to failing hearts. Eighty percent (80 %) of RV muscles from non-failing hearts (8/10) showed triggered activity compared to ≈ 33% (4/12) of their failing counterparts. Similarly, ≈ 63 % of LV muscles from non-failing hearts (5/8) exhibited arrhythmic behavior compared to 25 % (3/12) of those from failing hearts (Figure 1).

In the presence of isoproterenol/caffeine, arrhythmias in the form of extra systolic contractions with either high (Figure 2A and 2B) or low (Figure 2C and 2D) amplitudes were triggered in trabeculae isolated from both RV and LV of non-failing hearts, respectively. Similarly, isoproterenol/caffeine treatment resulted in triggered arrhythmia with either high (Figure 2E and 2F) or low (Figure 2G and 2H) amplitudes in trabeculae isolated from both RV and LV of failing hearts, respectively. Zacopride, added within 1 to 3 minutes after development of arrhythmia, was able to suppress these triggered activities of variant amplitudes in all susceptible muscles. Zacopride showed these effects in a concentration-dependent manner as is shown in Figure 2C, D, G and H. Calculated median inhibitory concentrations (IC_{50s}) of zacopride in the RV and LV from non-failing hearts are 40.4 and 30.3 μmol/L, respectively (Figure 2I). However, calculated IC_{50s} of zacopride in the RV and LV from failing hearts are 28.7 and 31.7 μmol/L, respectively (Figure 2J).

Isoproterenol/caffeine treatment in cardiac trabeculae from RV of failing hearts that were unsusceptible to its arrhythmogenic effects caused an increase in the Fdev (42.8 ± 7.1 mN/mm²), which was not quite significant (P = 0.0596) compared to basal Fdev (20.0 ± 3.6 mN/mm²) (Figure 3A). However, it resulted in significant decreases in contractile kinetic parameters, including TTP (166 ± 7 ms; P < 0.01) and RT50 (137 ± 9 ms; P < 0.05) compared to their values at basal conditions (TTP: 225 ± 10 ms and RT50: 178 ± 14 ms) (Figure 3B & C), respectively. Zacopride in a concentration range of 5 to 100 μmol/L did not significantly change the Fdev, contractile kinetic parameters or induce any kind of arrhythmia in the presence of isoproterenol/caffeine (Figure 3A – D). Equally, isoproterenol/caffeine treatment in cardiac trabeculae from LV of failing hearts that were unsusceptible to its arrhythmogenic effects caused an insignificant increase in the Fdev (19.6 ± 4.3 mN/mm²) compared to its basal value (10.7 ± 2.8 mN/mm²) (P = 0.6610) (Figure 3E). Nonetheless, it resulted in significant decreases in contractile kinetic parameters, including TTP (177 ± 9 ms; P < 0.05) and RT50 (103 ± 5 ms; P < 0.01) compared to their values at basal conditions (TTP; 222 ± 12 ms and RT50; 140 ± 8 ms) (Figure 3F & G), respectively. Again, zacopride in the same concentration range did not significantly change the Fdev, contractile kinetic parameters or induce any kind of arrhythmia in the presence of isoproterenol/caffeine (Figure 3E – H). Increasing zacopride concentration up to 200 μmol/L did not change the contractile profile or induce any kind of arrhythmia in a trabecula from the LV of a failing heart under the same conditions (Supplementary Figure 2).

Further, we investigated the direct effects of zacopride (5 – 200 μmol/L) on contractile profiles of trabeculae isolated from both non-failing and failing human hearts. In cardiac trabeculae from RV of non-failing hearts, zacopride resulted in a concentration-dependent

decrease in the F_{dev} , which reached significance at 100 $\mu\text{mol/L}$ ($13.9 \pm 2.2 \text{ mN/mm}^2$; $P < 0.05$) and 200 $\mu\text{mol/L}$ ($7.4 \pm 1.7 \text{ N/mm}^2$; $P < 0.01$) compared to basal F_{dev} ($24.4 \pm 3.0 \text{ mN/mm}^2$) (Figure 4A). However, there were no significant differences in the TTP or RT50 at all investigated concentrations of zacopride compared to their values at basal conditions (Figure 4B & C, respectively). Surprisingly, zacopride at a concentration of 200 $\mu\text{mol/L}$ only resulted in contractile alternans as demonstrated in Figure 4D. Likewise, cardiac trabeculae from LV of non-failing hearts exhibited a concentration-dependent decrease in the F_{dev} but it did not reach significance ($P = 0.7719$) (likely due to low n , $n = 4$) (Figure 4E). Also, there were no significant differences in all contractile kinetic parameters, including TTP (Figure 4F) and RT50 (Figure 4G) at all investigated concentrations of zacopride compared to their values at basal conditions. Besides, we did not notice any contractile alternans in these muscles at high zacopride concentration of 200 $\mu\text{mol/L}$ as revealed in Figure 4H. On the other hand, cardiac trabeculae from the RV of failing hearts demonstrated a concentration-dependent decrease in the F_{dev} but it did not reach significance ($P = 0.1972$) (Figure 5A). Also, there were no significant differences in all contractile kinetic parameters, including TTP (Figure 5B) and RT50 (Figure 5C) at all investigated concentrations of zacopride compared to their values at basal conditions. Again, zacopride caused contractile alternans in 5 out of the 7 investigated RV trabeculae; 4 as expected at 200 $\mu\text{mol/L}$ (Figure 5D) and one appeared earlier at 10 μM . Likewise, cardiac trabeculae from the LV of failing hearts exhibited a concentration-dependent decrease in the F_{dev} but it did not reach significance ($P = 0.2200$) (Figure 5E). Also, there were no significant differences in all contractile kinetic parameters, including TTP (Figure 5F) and RT50 (Figure 5G) at all investigated concentrations of zacopride compared to their values at basal conditions. In contrast to trabeculae from the LV of non-failing hearts that did not show any contractile alternans, 2 out of 6 trabeculae from the LV of failing hearts exhibited contractile alternans at 200 $\mu\text{mol/L}$ (Figure 5H). In summary, 60 % of RV muscles from non-failing hearts (3/5) showed contractile alternans compared to ≈ 71 % (5/7) of their failing counterparts at 200 $\mu\text{mol/L}$ of zacopride. Yet, none of the LV muscles (0/4) from non-failing hearts exhibited contractile alternans compared to 33 % (2/6) of those from failing hearts (Figure 6).

Discussion

Generally, antiarrhythmic drugs have had restricted success and can occasionally trigger arrhythmia [23]. Even with the recent success of implantable defibrillators and the promising future of gene therapy, there is still an imperative need for novel antiarrhythmic drugs. A better understanding of cardiac ion channels and new ways to target selection and compound screening will grant innovative prospects for future drug discovery [23]. Recently, the moderate I_{K1} channel activator zacopride was shown to suppress triggered VT in rats [15]. Nonetheless, concerns were raised about the possibility of pro-arrhythmic activity after I_{K1} channel stimulation based on a promising anti-arrhythmic strategy of I_{K1} blockade in other animal models [16, 17]. Translation of results obtained in animal models to human pathophysiology is often hampered by species-dependent differences. For instance, humans exhibit lower repolarization reserve contributions from I_{K1} and I_{Ks} compared to dogs [18]. Also, the rat ventricle lacks functional I_{Kr} and I_{Ks} [24]. In addition, the extreme dissimilarity of electrical substrate in mouse/rat hearts, including the lack of any

appreciable AP plateau phase, very rapid heart rate and potentially unstable RP, may cause a negligible role for I_{K1} compared to larger hearts with slower heart rates, and stable AP plateaus, as humans possess [25]. Therefore, the goal of the current study was to investigate the *ex-vivo* effects of zacopride on triggered arrhythmia and contractility in ventricular human myocardium in order to validate data that was solely obtained from animal models.

Triggered arrhythmias originate from DADs that are most frequently observed under conditions of SR Ca^{2+} overload, where the high SR Ca^{2+} content can cause spontaneous Ca^{2+} release after the termination of the AP due to the activation of Na^+/Ca^{2+} exchange (NCX), and the decline of I_{K1} in both animals [26, 27] and humans [13, 28, 29]. In this regard, caffeine allows for increases in the open probability of the RyR2 without depleting the SR, and it decreases the threshold for SR Ca^{2+} release [30, 31]. On the other hand, isoproterenol increases the SR Ca^{2+} content to a high enough level to reach the threshold for SR Ca^{2+} release [32]. Our recent data showed a synchronization mechanism that exists for the generation of triggered activity in multicellular cardiac preparations of genetically mutated murine myocardium [33] and wild-type rat hearts under conditions of Ca^{2+} dysregulation induced either by isoproterenol (100 nmol/L) alone [33], or a combination of isoproterenol (100 nmol/L) and caffeine (0.5 mmol/L) (unpublished). In the current study, the same concentrations of isoproterenol/caffeine combination led to triggered arrhythmia in isolated cardiac muscles from both non-failing and end-stage failing hearts. However, the occurrence of arrhythmia in the RV and LV muscles of non-failing hearts was markedly higher than those of end-stage failing hearts. Consistent with these results, it has been reported that myocardial catecholamines' diminution and myocardial β -adrenergic receptors' down-regulation may elucidate tolerance of patients with severe congestive HF to VT as well as intolerance to conventional antiarrhythmic drugs [34]. Similarly, previous data indicate that myocardium from failing human heart is less sensitive to stimulation by isoproterenol than normal myocardium [35]. Furthermore, isoproterenol-induced inhibition of I_{K1} has been shown to be significantly reduced in human ventricular myocytes from the failing heart compared to the non-failing heart [13]. In contrast, it has been revealed that residual β -adrenergic responsiveness conspires to significantly augment the susceptibility to triggered arrhythmias in HF through I_{K1} down-regulation, NCX upregulation and SR Ca^{2+} overload-induced spontaneous SR Ca^{2+} release and after contractions in rabbits [26, 27]. Nonetheless, the authors of these studies stated that preserved β -adrenergic responsiveness that may take place only in HF that is not end-stage [36] is an essential contributor to arrhythmogenicity in HF [27]. In end-stage human HF, there are fewer sudden arrhythmic deaths likely due to the entire loss of β -adrenergic responsiveness [34, 35]. During this stage, susceptibility for arrhythmia may be lower, as pump failure continues, due to the inability of SR Ca^{2+} load to reach a level that is high enough for spontaneous SR Ca^{2+} release even though increased NCX and decreased I_{K1} may persist [26]. This clearly confirms the lower incidence rate of triggered arrhythmia in muscles of end-stage failing hearts compared to those of non-failing hearts in this study.

Zacopride is a moderate I_{K1} channel stimulator that selectively amplifies the I_{K1} current up to 40%, through the activation of the Kir2.1 homomeric channel via a PKA-dependent pathway [15, 37]. This modest enhancement in I_{K1} is different from that induced by the I_{K1} channel gene-transfer strategy, where the I_{K1} is increased by more than 100%, which makes

zacopride a promising antiarrhythmic candidate [9, 15]. Importantly, zacopride has been established to suppress both *ex-vivo* and *in-vivo* induced triggered arrhythmia in rats [15]. Consistently, our current data demonstrate the ability of zacopride to eliminate the *ex-vivo* triggered arrhythmia in muscles from both non-failing and failing human hearts in a concentration-dependent manner, with an effective IC_{50} in the range of 28 to 40 $\mu\text{mol/L}$. In line with these effective high zacopride concentrations, a previous study showed that mandatory zacopride concentration to attain a maximal efficacy for the I_{K1} activation is greatly elevated in human embryonic kidney-293 cells (100 $\mu\text{mol/L}$) [37] compared to rat ventricular myocytes (1 $\mu\text{mol/L}$) [15]. Moreover, zacopride (at the highest applicable concentration, 200 $\mu\text{mol/L}$) did not affect isoproterenol-induced cardiac contractile changes, including F_{dev} , TTP, RT50 and RT90 in the majority of failing heart muscles, which were not susceptible to the arrhythmogenic effect of isoproterenol/caffeine combination. In addition, zacopride did not induce any arrhythmia in the presence of isoproterenol/caffeine on these muscles. Similarly, application of K^+ channel openers inhibited isoproterenol-induced after contractions in both guinea-pig and human myocytes. Also, these K^+ channel openers returned the contraction to a point close to or higher than those with isoproterenol alone, but did not change the isoproterenol-induced alterations in TTP or RT50 and did not repeat the appearance of after contractions [38]. From this study the authors concluded that the isoproterenol inotropic and lusitropic effects are separable from its arrhythmogenic effects. Additionally, the inability of K^+ channel openers to antagonize these isoproterenol-induced contractile changes may imply their independency of PKA-dependent pathways [38]. Thus, they proposed different mechanisms regarding these drug effects, which include: 1) AP shortening could diminish the possibility of L-type Ca^{2+} channel reactivation, which might be an underlying mechanism in the development of arrhythmia, 2) a shorter plateau region may possibly cause less Ca^{2+} influx due to faster calcium current deactivation, as a result free $[Ca^{2+}]_i$ and following SR loading might be minimized and 3) in the presence of short AP plateau NCX Ca^{2+} efflux may be more favored compared to NCX Ca^{2+} influx, which mainly occurs with a longer AP [38]. This may explain the similar effects of zacopride on isoproterenol/caffeine-induced arrhythmia and contractile changes; nevertheless, it requires further investigation, particularly, due to other reports which show that I_{K1} in human ventricular myocytes can be inhibited by isoproterenol-induced PKA-mediated phosphorylation [13].

Although I_{K1} enhancement to stabilize RP is beneficial, especially in the treatment of triggered arrhythmia, it could also be harmful because of the reduction in excitability, propagation rate, and AP duration [26]. In the absence of isoproterenol/caffeine, zacopride led to a negative inotropic effect in a concentration-dependent manner. Reduced cardiac contraction was observed only at high zacopride concentration of 200 $\mu\text{mol/L}$, along with the occurrence of cardiac alternans in muscles from both non-failing (RV) and failing (RV & LV) human hearts. Likewise, K^+ channel openers that returned the contraction to a point close to or higher than those with isoproterenol alone [38] resulted in significant shortening of cardiac APD and reduction of the twitch amplitude in the absence of isoproterenol [39]. Furthermore, it has been reported that AP shortening is responsible for the negative inotropic effect of nifedipine in neonatal cardiomyocyte due to reduction in activator Ca^{2+} influx through NCX [40]. On the other hand, alternans is a risk factor for cardiac arrhythmia, and

the main mechanism underlying alternans resides in a disturbance of Ca^{2+} signaling [41] as well as in recirculation of Ca^{2+} between heart beats [42]. However, the actual mechanism for these zacopride-induced contractile defects and alternans needs to be assessed.

Of note, in this study there are some differences in the responses of muscles obtained from the RV and LV of both non-failing and failing hearts. Mostly, the RV muscles demonstrated higher responses to isoproterenol/caffeine and/or zacopride compared to LV muscles. The RV and LV encompass diverse embryologic, structural, metabolic, and electrophysiologic features, which could explain the difference between RV and LV in these experimental responses [43]. For example, repolarizing K^+ currents I_{TO1} and I_{Ks} , but not I_{K1} are larger in RV than LV of canine myocardium [44]. Also, RV of canine myocardium exhibited enhanced β -adrenergic responsiveness compared to the LV [43].

Conclusions

Under arrhythmogenic conditions, zacopride shows promising antiarrhythmic effects against triggered arrhythmia induced by isoproterenol/caffeine in ventricular human myocardium. Interestingly, this occurred with zacopride IC_{50} concentration range, most likely, devoid of contractile side effects, which gives this drug a higher chance to be effectively used in the treatment of human ventricular tachyarrhythmia, yet, applicable dosage should be carefully determined. In the absence of Ca^{2+} overload/arrhythmia induced by isoproterenol/caffeine, zacopride, albeit at high concentrations, decreases the force of contraction and increases likelihood of occurrence of contractile alternans, which may predispose the heart to contractile dysfunction and/or arrhythmia, especially in those who are using the drug at high doses as an anti-emetic, gastroprokinetic or anxiolytic agent. Overall, our results represent a key step in translating zacopride from the benchtop to the bedside in the research area to truly investigate the potential *in-vivo* effects of this drug on both arrhythmia and contractility in human hearts.

Study Limitations

The inability of zacopride to affect the force of contraction or induce arrhythmia in the presence of Ca^{2+} overload could signify a differential mechanism of action for this drug in the heart, yet, this needs to be elucidated. In addition, the ability of this drug to directly induce contractile dysfunction and cardiac alternans requires further assessment. Due to time-constraints in using live human tissue and the limited number of experimental protocols that can be completed on such intact muscles, this study focused on the contractile aspects, and contractile read-outs of arrhythmic behavior. The effects of zacopride on AP as well as on Ca^{2+} transients in susceptible human heart muscles are important factors that remain to be investigated to further elucidate the antiarrhythmic mechanism of this drug.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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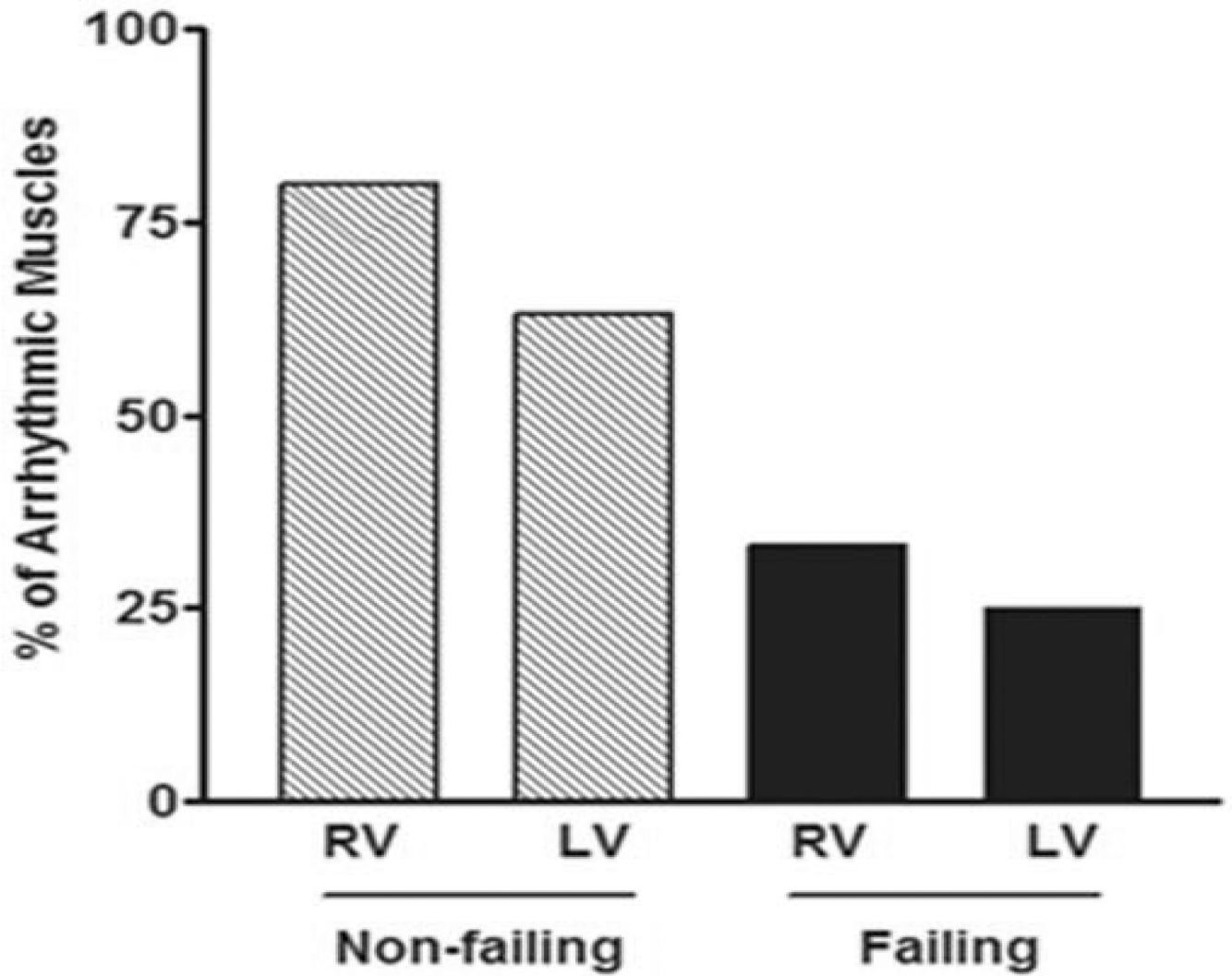


Figure 1. Percentage of arrhythmia occurrence in ventricular trabeculae isolated from non-failing [RV: right ventricle (8 out of 10) & LV: left ventricle (5 out of 8)] and failing [RV (4 out of 12) & LV (3 out of 12)] human hearts.

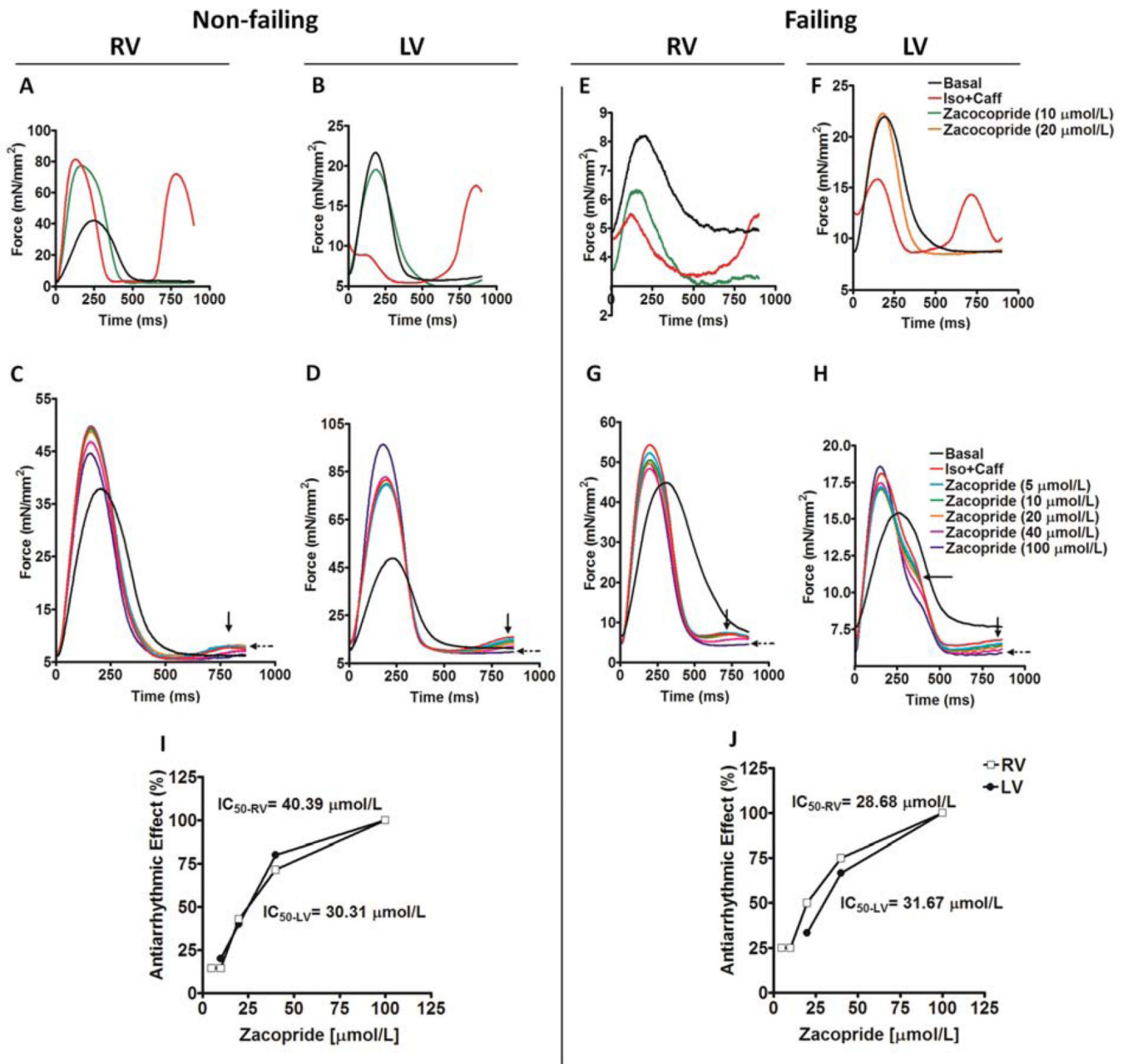


Figure 2. Original representative recordings of twitches (normalized to cross-sectional area) in non-failing human myocardium at basal status, in presence of Iso + Caff [isoproterenol (100 nmol/L)/caffeine (0.5 mmol/L)] and zacopride effective antiarrhythmic concentrations when arrhythmias in the form of extra systolic contractions with either high (A & B) or low (C & D) amplitudes are triggered in trabeculae isolated from both RV, right ventricle (heart # 415217 & 514489) and LV, left ventricle (heart # 219852 & 514489) of non-failing hearts, respectively. Original representative recordings of twitches (normalized to cross-sectional area) in failing human myocardium at basal status, in presence of Iso + Caff and zacopride effective antiarrhythmic concentrations when arrhythmias in the form of extra systolic

contractions with either high (**E & F**) or low (**G & H**) amplitudes are triggered in trabeculae isolated from both RV (heart # 233587 & 645444) and LV (heart # 728878 & 645444) of failing hearts, respectively. IC_{50} : median inhibitory concentrations of zacopride in the RV and LV of non-failing (**I**) and failing (**J**) human hearts.

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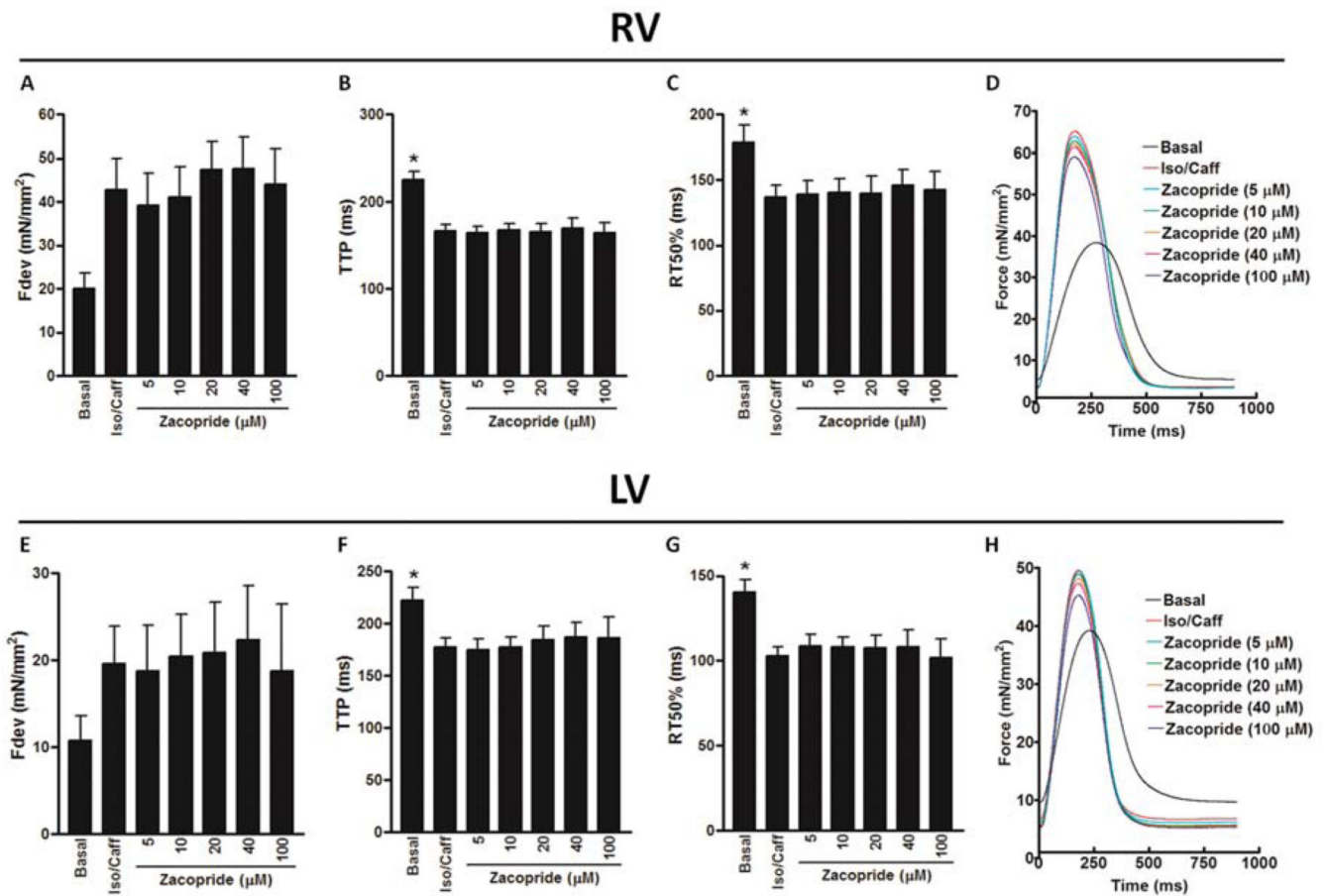


Figure 3.

The effect of zacopride on Iso/Caff [isoproterenol (100 nmol/L)/caffeine (0.5 mmol/L)]-induced changes in contractility of ventricular trabeculae isolated from the RV, right ventricle of failing human hearts, which are not susceptible to Iso/Caff-provoked triggered arrhythmia including: Fdev, peak isometric developed force (A); TTP, time to peak force (B); and RT50, time from peak force to 50% relaxation (C). Original representative recordings of twitches (normalized to cross-sectional area) in the RV of failing human myocardium (heart # 611422) that is not susceptible to Iso/Caff-provoked triggered arrhythmia at basal status, in presence of Iso/Caff and zacopride concentrations (5 – 100 μmol/L) (D). The effect of zacopride on Iso/Caff-induced changes in contractility of ventricular trabeculae isolated from the LV, left ventricle of failing human hearts, which are not susceptible to Iso/Caff-provoked triggered arrhythmia including: Fdev (E); TTP, time to peak force (F); and RT50 (G). Original representative recordings of twitches (normalized to cross-sectional area) in the LV of failing human myocardium (heart # 611422) that are not susceptible to Iso/Caff-provoked triggered arrhythmia at basal status, in presence of Iso/Caff and zacopride concentrations (5 – 100 μmol/L) (H). *A significant change compared to Iso/Caff, $P < 0.05$. $n = 4 - 9$ based on time-dependent increase in zacopride concentration.

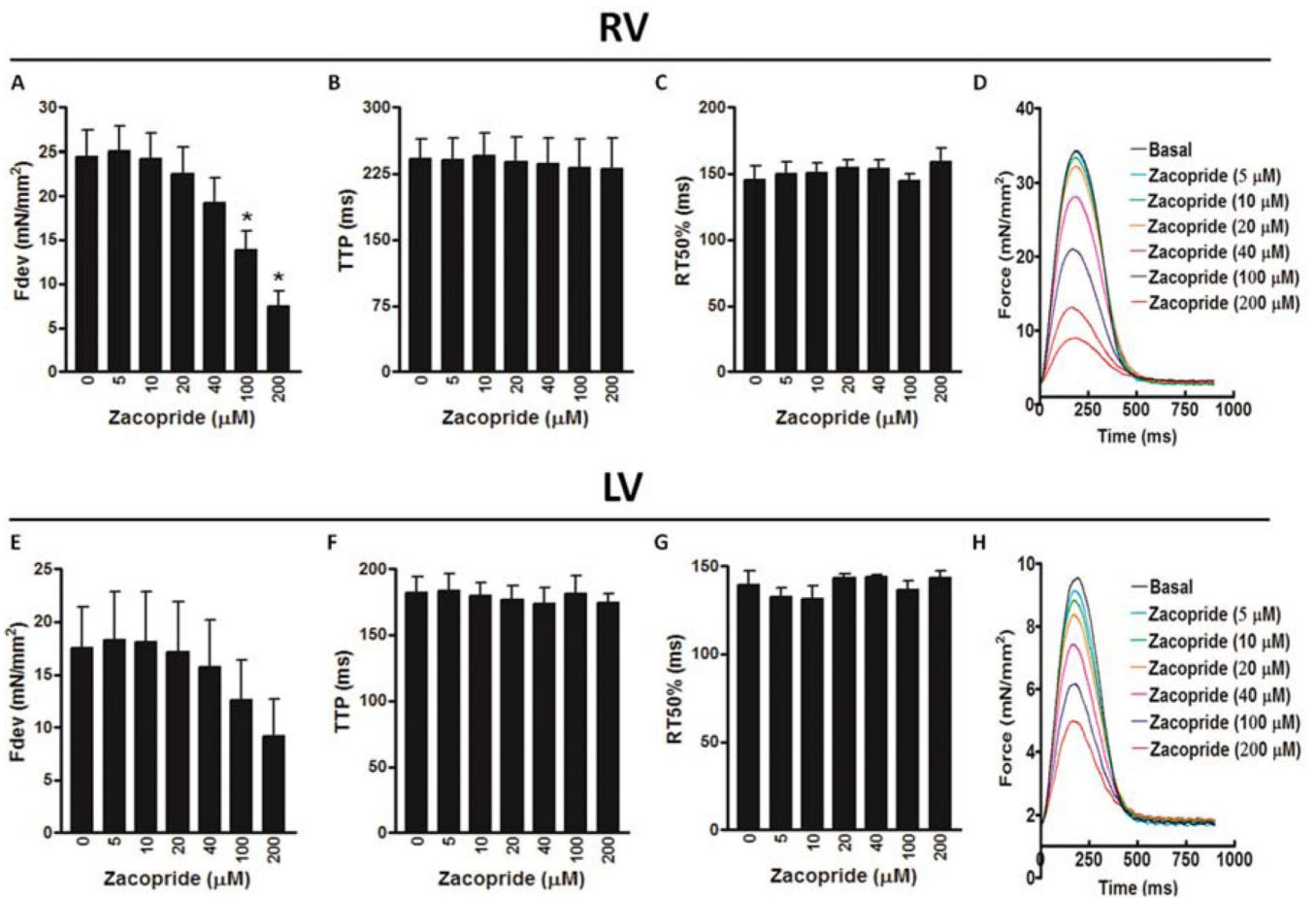
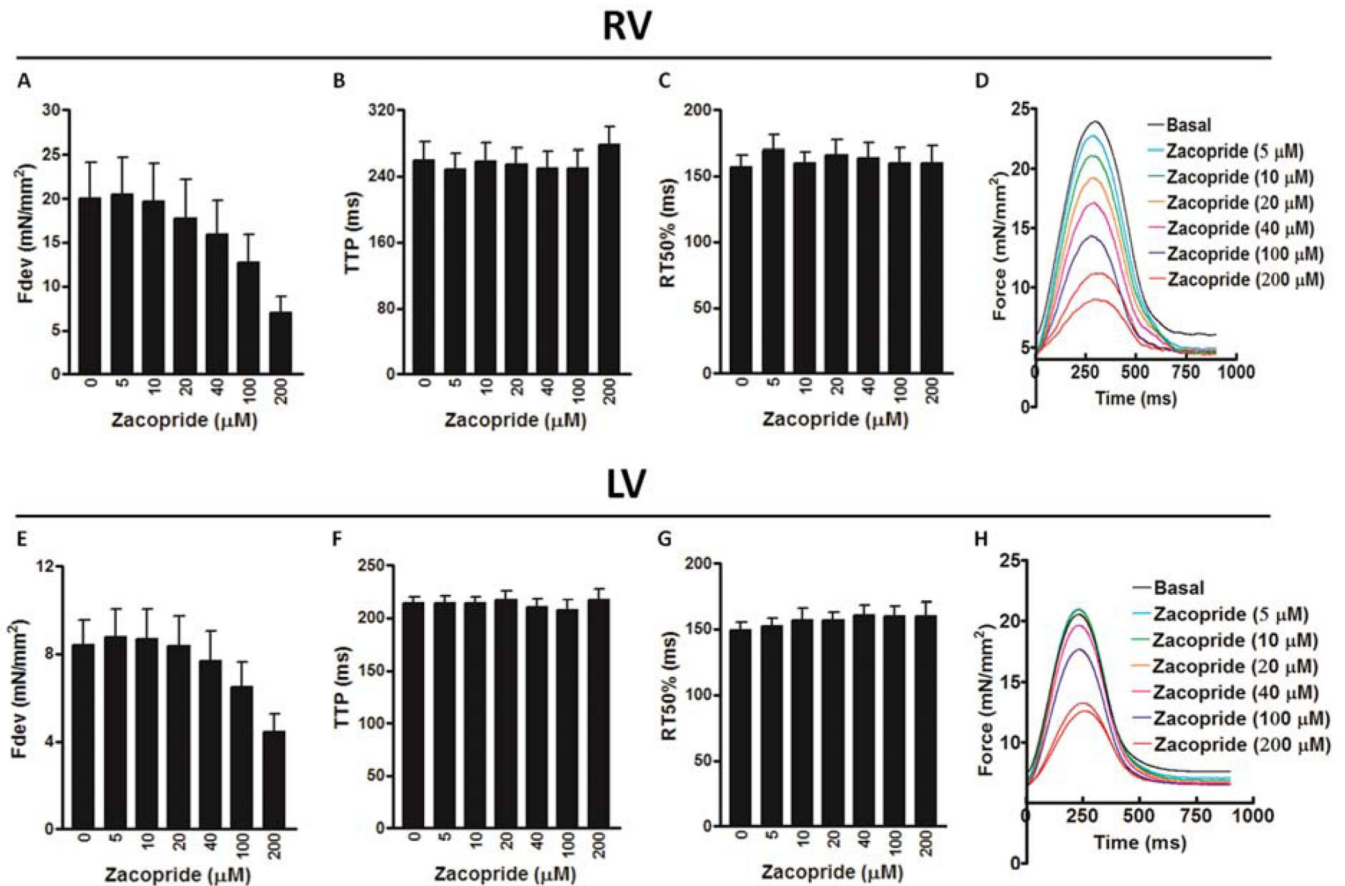


Figure 4.

The effect of zacopride on contractile parameters of ventricular trabeculae isolated from the RV, right ventricle of non-failing human hearts, including: Fdev, peak isometric developed force (A); TTP, time to peak force (B); and RT50, time from peak force to 50% relaxation (C). Superimposed original representative recordings of twitches (normalized to cross-sectional area) in the RV of non-failing human myocardium (heart # 958987) in the presence of zacopride (5 – 200 $\mu\text{mol/L}$) showing a concentration-dependent decrease in the Fdev and occurrence of contractile alternans at the highest applied concentration of 200 $\mu\text{mol/L}$ (D). The effect of zacopride on contractile parameters of ventricular trabeculae isolated from the LV, left ventricle of non-failing human hearts, including, Fdev (E), TTP (F) and RT50 (G). Superimposed original representative recordings of twitches (normalized to cross-sectional area) in the LV of non-failing human myocardium (heart # 958987) in the presence of zacopride (5 – 200 $\mu\text{mol/L}$) showing a concentration-dependent decrease in the Fdev (H).

*A significant change compared to basal (zacopride concentration: 0 $\mu\text{mol/L}$), $P < 0.05$ and $n = 4 - 5$.

**Figure 5.**

The effect of zacopride on contractile parameters of ventricular trabeculae isolated from the RV, right ventricle of failing human hearts, including: Fdev, peak isometric developed force (A); TTP, time to peak force (B); and RT50, time from peak force to 50% relaxation (C). Superimposed original representative recordings of twitches (normalized to cross-sectional area) in the RV of failing human myocardium (heart # 904475) in the presence of zacopride (5 – 200 μmol/L) showing a concentration-dependent decrease in the Fdev and occurrence of contractile alternans at the highest applied concentration of 200 μmol/L (D). The effect of zacopride on contractile parameters of ventricular trabeculae isolated from the LV, left ventricle of failing human hearts, including, Fdev (E), TTP (F) and RT50 (G). Superimposed original representative recordings of twitches (normalized to cross-sectional area) in the LV of non-failing human myocardium (heart # 820447) in the presence of zacopride (5 – 200 μmol/L) showing a concentration-dependent decrease in the Fdev and occurrence of contractile alternans at the highest applied concentration of 200 μmol/L (H). n = 5 – 7.

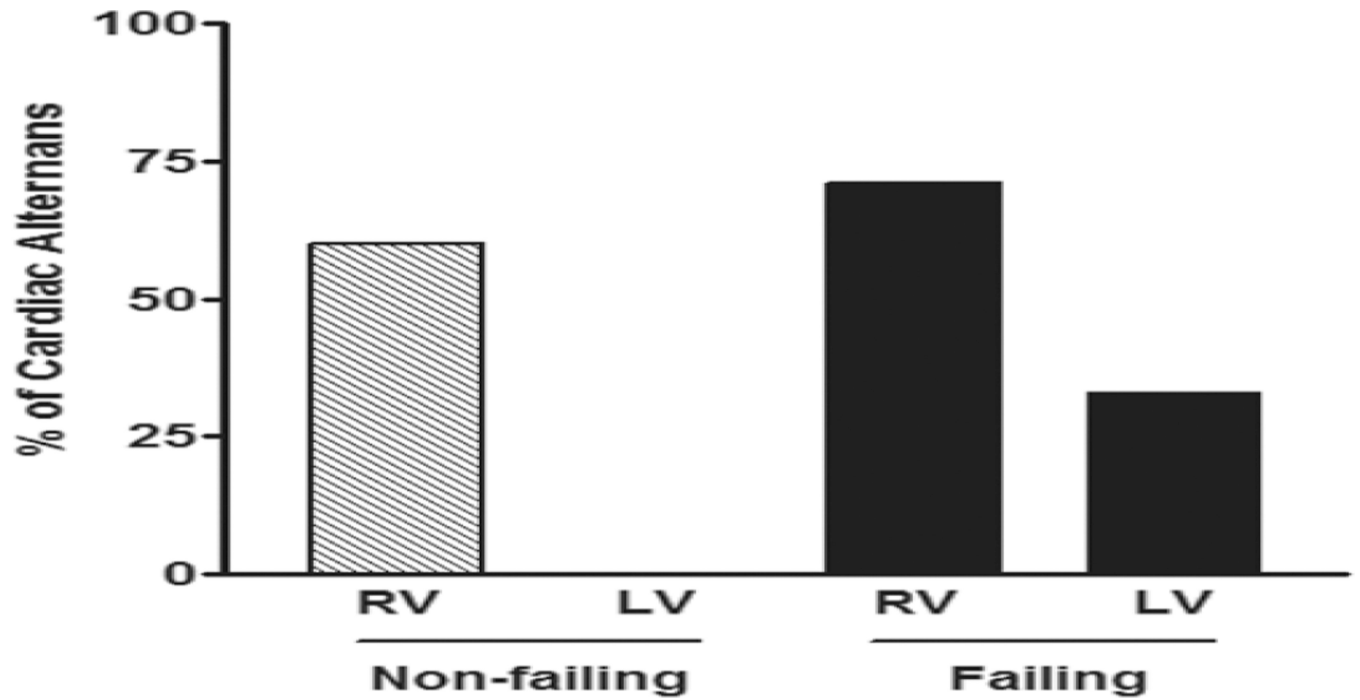


Figure 6. Percentage of the occurrence of contractile alternans in ventricular trabeculae isolated from non-filing [RV, right ventricle (3 out of 5) & LV, left ventricle (0 out of 4)] and failing [RV (5 out of 7) & LV (2 out of 6)] human hearts.

Table 1

Characteristics of Non-failing Hearts – Arrhythmia Experiments

Heart #	Age, yrs	Sex	Race	Cause of Death	LVEF, %	HR, BPM	Heart Weight, g	Arrhythmia After Iso/Caff	
								RV	LV
219852	30	F	Caucasian	CE/RF	55	135	299	No	Yes
331253	42	F	Hispanic	CVA/ICH	55	85	390	Yes	Yes
364587	19	M	Caucasian	Blunt Injury/MVA	25	131	300	Yes	NA
402879	54	M	Caucasian	ICB/ICH	NA	NA	474	Yes	No
415217	42	M	Caucasian	ICB/ICH	NA	NA	508	Yes	Yes
435578	20	M	Caucasian	DO/Anoxia	35	112	324	Yes	NA
514489	42	F	Caucasian	Cardiac Arrest/Anoxia	55	114	327	Yes	Yes
694855	46	F	Caucasian	ICH/CVA and SAH	60	94	356	Yes	Yes
785258	51	F	Caucasian	ICH/CVA	60	82	335	No	No
984478	54	F	African American	ICB/ICH	55	87	348	Yes	No

BPM, beat per minute; CE, cerebral edema; CVA, cerebral vascular accident; DO, drug overdose; F, female; HR, heart rate; ICB, intracerebral bleeding; ICH, intracerebral hemorrhage; Iso/Caff, Isoproterenol (100 nmol/L)/caffeine (0.5 mmol/L); LVEF, left ventricular ejection fraction; M, male; MVA, motor vehicle accident; NA, non applicable; RF, respiratory failure; SAH, subarachnoid hemorrhage; Yr, year.

Table 2

Characteristics of Failing Hearts – Arrhythmia Experiments.

Heart #	Age, yrs	Sex	Race	Etiology	Heart Weight, g	Arrhythmia After Iso/Caff	RV	LV
233587	65	M	Caucasian	ICM	692	Yes	Yes	No
369452	61	M	African American	NICM	540	No	No	No
390112	60	F	Caucasian	NICM	608	No	No	No
588415	52	M	Caucasian	NICM	390	No	No	No
599014	50	M	Caucasian	NICM	667	No	No	No
611422	68	M	Caucasian	ICM	576	No	No	No
631231	49	F	Asian	CCM	306	No	No	NA
645444	47	M	African American	ICM	411	Yes	Yes	Yes
728878	40	M	Caucasian/African American	NICM	747	Yes	Yes	Yes
736955	61	F	Caucasian	ICM	575	No	No	No
777902	59	M	Caucasian	CAD	530	Yes	Yes	NA
820447	68	M	Caucasian	NICM	567	NA	NA	No
935541	51	M	African American	NICM	453	NA	NA	Yes
971258	57	M	Caucasian	ICM	619	No	No	No

CAD, coronary artery disease; CCM, chemo-induced cardiomyopathy; F, female; ICM, ischemic cardiomyopathy; Iso/Caff, isoproterenol (100 nmol/L)/caffeine (0.5 mmol/L); M, male; NA, non applicable; NICM, ischemic cardiomyopathy; yr, year.

Table 3

Characteristics of Non-failing Hearts – Contractility Experiments

Heart #	Age, yr	Sex	Race	Cause of Death	LVEF, %	HR, BPM	Heart Weight, g
435578	20	M	Caucasian	DO/Anoxia	35	112	324
460025	69	F	Caucasian	CVA/ICH	60	109	435
925852	34	F	African American	RF/Anoxia	50	82	672
958987	40	M	Caucasian	DO/Cardiac arrest	60	113	615
987692	34	F	Caucasian	MVA/Trauma	30	112	313

BPM, beat per minute; CVA, cerebral vascular accident; DO, drug overdose; F, female; HR, heart rate; LVEF, left ventricular ejection fraction; M, male; MVA, motor vehicle accident; RF, respiratory failure; Yr, year.

Table 4

Characteristics of Failing Hearts – Contractility Experiments

Heart #	Age, yr	Sex	Race	Etiology	Heart Weight, g
679533	47	M	Caucasian	NICM	930
777902	59	M	Caucasian	CAD	530
806554	39	F	African American	NICM	365
820447	68	M	Caucasian	NICM	567
904475	35	F	Caucasian	PPCM	262
497522	62	F	Caucasian	NICM	649

CAD, coronary artery disease; F, female; Iso/Caff, Isoproterenol (100 nmol/L)/caffeine (0.5 mmol/L); M, male; NICM, ischemic cardiomyopathy; PPCM, post partum cardiomyopathy; yr, year.