Antiarrhythmic and cardiac electrophysiological effects of SZV-270, a novel compound with combined Class I/B and Class III effects, in rabbits and dogs

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Abstract

Cardiovascular diseases are the leading causes of mortality. Sudden cardiac death is most commonly caused by ventricular fibrillation (VF). Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia and a major cause of stroke and heart failure. Pharmacological management of VF and AF remains suboptimal due to limited efficacy of antiarrhythmic drugs and their ventricular proarrhythmic adverse effects. In this study, the antiarrhythmic and cardiac cellular electrophysiological effects of SZV-270, a novel compound, were investigated in rabbit and canine models. SZV-270 significantly reduced the incidence of VF in rabbits subjected to coronary artery occlusion/reperfusion, reduced the incidence of burst-induced AF in a tachypaced conscious canine model of AF. SZV-270 prolonged frequency corrected QT interval, lengthened action potential duration and effective refractory period in ventricular and atrial preparations and blocked I_{Kr} in isolated cardiomyocytes (Class III effects), reduced maximum rate of depolarization (V_{max}) at cycle lengths smaller than 1000 ms in ventricular preparations (Class I/B effect). Importantly, SZV-270 did not provoke Torsades de Pointes arrhythmia in an anesthetized rabbit proarrhythmia model characterized by impaired repolarization reserve. In conclusion, SZV-270 with its combined Class I/B and III effects can prevent re-entry arrhythmias with reduced risk of provoking drug-induced Torsades de Pointes.

Keywords

action potential duration, atrial fibrillation, combined Class I/b and Class III effect, ventricular fibrillation, Torsades de Pointes

List of abbreviations

AERP: right atrial effective refractory period

AF: atrial fibrillation

APA: action potential amplitude

APD_{50}: action potential duration at 50% of repolarization

APD_{90}: action potential duration at 90% of repolarization

I_{Ca}: voltage-dependent calcium current

I_{K1}: inward rectifier potassium current

I_{Kr}: rapidly activating delayed rectifier potassium current

I_{Ks}: slowly activating delayed rectifier potassium current

I_{to}: transient outward potassium current

MABP: mean arterial blood pressure

QTc: frequency corrected QT interval

RMP: resting membrane potential

TdP: Torsade de Pointes polymorphic ventricular tachycardia

SEM: standard error of the mean

$STV_{QT:}$ short-term variability of the QT interval

VF: ventricular fibrillation

V_{max}: maximum rate of the depolarization

Introduction

Cardiovascular diseases remain the leading causes of mortality in the developed world. Approximately 18 million lives are lost annually due to sudden cardiac death, most commonly caused by severe ventricular arrhythmias degenerating into ventricular fibrillation (VF) (Shomanova et al., 2020). Following the significant setbacks for pharmacological prevention of ventricular arrhythmias that were provided by the Cardiac Arrhythmia Suppression Trials (The Cardiac Arrhythmia Suppression Trial Investigators, 1989; The Cardiac Arrhythmia Suppression Trial II Investigators, 1992) and the Survival with Oral D-Sotalol trial (Waldo et al., 1996), where sodium channel blocker Class I/C and potassium channel blocker Class III compounds - instead of improving clinical outcome - increased mortality in post-myocardial infarction patients with reduced ejection fraction, the attention shifted towards potential new antiarrhythmic drugs with more complex ion channel and receptor modulatory effects.

Atrial fibrillation (AF), the most prevalent sustained cardiac arrhythmia (Kannel et al., 1982; Andrade et al., 2014), is associated with significant morbidity and mortality, leading to stroke (Lip et al., 2011) and heart failure (Larned and Laskar, 2009). The therapy of AF is not optimal, since pharmacological therapy has limited efficacy (Andrade et al., 2014) and antiarrhythmic drugs exhibit marked proarrhythmic potential due to their cardiac ventricular electrophysiological adverse effects (Fenichel et al., 2004), while AF ablation can lead to complications (Andrade et al., 2014; Aksu et al., 2019; Friedman et al., 2020) and recurrence of AF following ablation also occurs (Takigawa et al., 2017).

One promising approach to safer and more effective pharmacological arrhythmia management is the use novel compounds that exhibit more complex actions and modulate several ionic currents. Indeed, amiodarone, a compound affecting a several ionic currents, remains one of the most effective antiarrhythmic drugs both for the management of AF and

severe ventricular arrhythmias (Mujovic et al., 2020), however, especially during its chronic application, it exhibits severe extracardiac adverse effects (Hilleman et al., 1998; Mujović, 2020). Class III antiarrhythmic drugs prolong myocardial repolarization and can effectively reduce re-entry arrhythmias (Hashimoto et al., 1995; Hohnloser et al., 1995; Fei and Frame, 1996), however, they can also provoke Torsades de Pointes (TdP) tachycardia (Verduyn et al., 1997) and D-sotalol increased mortality in post-myocardial infarction patients (Waldo et al., 1996). Despite its significant QT prolonging effect, amiodarone has a relatively low torsadogenic adverse effect (Hohnloser et al., 1994; Belardinelli et al., 2003; Thomsen et al., 2004), possibly due to decreased transmural dispersion of repolarization and inhibition of early afterdepolarization (EAD) formation following amiodarone administration (Sicouri et al., 1997), similarly to Class I/B antiarrhythmic drugs (Shimizu and Antzelevitch, 1997; Assimes and Malcolm, 1998). Therefore, the development of novel compounds with complex actions exhibiting combined Class I/B and Class III effects and devoid of severe extracardiac adverse effects, that are effective against both supraventricular and ventricular arrhythmias, is justified.

In this study, a novel compound with complex actions, SZV-270 (**Fig. 1**), was investigated regarding its cardiac cellular electrophysiological effects in rabbit and canine atrial and ventricular preparations. The ventricular antiarrhythmic effects of SZV-270 were also investigated in rabbits subjected to coronary artery occlusion/reperfusion, and its effects on atrial fibrillation were tested in dogs with chronic atrial tachypacing-induced atrial remodeling. Importantly, the potential proarrhythmic adverse effects of SZV-270 were also studied in a rabbit model developed by our laboratory (Lengyel et al., 2007).

Materials and methods

Ethical issues

All animal care and the described experiments complied with the Guide for the Care and Use of Laboratory Animals (U.S.A. NIH publication No 85-23, revised 1996) and conformed to the the Directive 2010/63/EU of the European Parliament. The experimental protocols had been approved by the Ethical Committee for the Protection of Animals in Research of the University of Szeged, Szeged, Hungary (I-74-18-2016; I-74-15/2017; I-74-24/2017); and also by the Department of Public Health and Food Chain Safety at the Csongrád County Government Office (XIII/4227/2016; XIII/3330/2017; XIII/3331/2017).

Coronary artery occlusion/reperfusion induced ventricular arrhythmias in rabbits

Coronary artery occlusion/reperfusion induced arrhythmias were studied in pentobarbitone (30 mg/kg) anesthetized male rabbits (weighing 2 to 3 kg, n=10/group) as described previously (Baczko et al., 2000). Briefly, thoracotomy was performed in the fourth intercostal space and artificial ventilation was performed (Harvard rodent ventilator, model 683, Harvard Apparatus, South Natick, MA, USA). Following pericardiotomy, a loose loop of 4-0 atraumatic silk (Ethicon, Edinburgh, UK) was placed around the first branch of the left circumflex coronary artery, just under its origin. After a 15-min stabilization of blood pressure and heart rate, saline or 0.3 mg/kg SZV-270 was administered i.v. during a 1 min infusion in a volume of 2 ml/kg, 5 min prior to coronary artery occlusion. Coronary artery occlusion and local myocardial ischaemia was produced by tightening the loose loop. After 10 min of coronary artery occlusion, the ligature was released to permit reperfusion for 10 min.

The The ECG was recorded using subcutaneous needle electrodes (lead I, II, III), was digitized and stored on a computer for off-line analysis using National Instruments data acquisition hardware (National Instruments, Austin, Texas, USA) and SPEL Advanced Haemosys software (version 3.2, MDE Heidelberg GmbH, Heidelberg, Germany). The frequency corrected QT interval (QTc) was calculated by a formula specifically worked out for anaesthetized rabbits (Batey and Coker, 2002), as follows: QTc = QT – (0.704 * (RR-250)). Arrhythmias were diagnosed in accordance with the revised Lambeth conventions as ventricular tachycardia, ventricular fibrillation and other types of arrhythmias including single extrasystoles, bigeminy, salvos and bradycardia (Curtis et al., 2013).

Proarrhythmia studies in rabbits

To test whether SZV-270 had proarrhythmic adverse effects, an *in vivo* rabbit model of Torsades de Pointes (TdP) was used, developed by our laboratory (Lengyel et al., 2007). Rabbits of both sexes (weighing 2-3 kg; n=8-11/group) were anaesthetized with thiopentone (50 mg/kg). A catheter filled with isotonic saline containing 500 IU/mL heparin was inserted into the left carotid artery for the measurement of arterial blood pressure and the right jugular vein was cannulated for i.v. drug administration. The animals were allowed to stabilize for 15 min and baseline measurements were taken. The first group of rabbits received the I_{Kr} blocker dofetilide (25 μg/kg) in a volume of 2 mL/kg in a 5-min infusion. The second group was administered a combination of the I_{Ks} blocker HMR1556 (Gögelein et al., 2000) in 0.1 mg/kg and 25 μg/kg dofetilide. The third group received a combination of 0.1 mg/kg HMR1556 and 0.3 mg/kg SZV-270. The electrocardiograms were recorded and arrhythmias were diagnosed as described in the previous section.

In order to characterize the instability of beat-to-beat repolarization, Poincaré plots of the QT intervals were constructed where each QT value was plotted against its former value, using

31 consecutive QT interval measurements in sinus rhythm at a given time point during the experiments. The beat-to-beat short-term variability of QT intervals (STV_{QT}) was calculated using the following formula: $STV = \sum|D_{n+1}-D_n| \ (30x\sqrt{2})^{-1}$, where D is the duration of the QT interval. The STV_{QT} has been shown in experimental and clinical settings to be a better predictor of the development of severe ventricular arrhythmias than QT prolongation (Lengyel et al., 2007; Hinterseer et al., 2010).

Atrial fibrillation following chronic atrial tachypacing in conscious dogs

Atrial fibrillation was induced in male Beagle dogs (n=6) weighing 12-15 kg as described previously (Baczko et al., 2014). In brief, two bipolar pacemaker electrodes (Synox SX 53-JBP and Synox SX 60/15-BP, Biotronik Hungary Ltd., Hungary) were implanted into the right atrial appendage and the apex of the right ventricle were connected to pacemakers (Logos DS and Philos S, Biotronik Hungary Ltd., Hungary) placed in subcutaneous pockets in the neck area. The implantation was followed by radiofrequency catheter ablation of the AV node. Following a 5-day recovery from surgery, right atrial tachypacing was started at 400 beats/min (ICS 3000 Programmer, Biotronik Hungary Ltd., Hungary), maintained for 6 weeks before the experiments to induce atrial electrical remodeling (monitored by the measurement of the right atrial effective refractory period (AERP) every second day). The AERPs were measured at basic cycle lengths (BCL) of 300 ms with a train of 10 stimuli (S1) followed by an extrastimulus (S2), with the AERP defined as the longest S1-S2 interval that did not produce a response.

On the day of the experiment atrial pacing was stopped, continuous recording of the electrocardiogram started using precordial leads and the AERP was measured. A control set of 10-second-long rapid atrial bursts (25 times, 800 beats/min, at twice threshold) were performed to induce atrial fibrillation in conscious dogs preceded by an infusion of vehicle in 15 min. Following the measurement of AERP, additional sets of atrial bursts were applied subsequent

to SZV-270 (0.3 mg/kg), or dofetilide (Sigma-Aldrich, 25 μg/kg), i.v. administration. At least 5 days were allowed for washout between *in vivo* experiments with the two compounds. Intravenous infusions were performed using a programmable infusion pump (Terufusion TE-3, Terumo Europe, Leuven, Belgium). The ECG was recorded using precordial leads, using SPEL Advanced Haemosys software (version 3.2, MDE Heidelberg GmbH, Heidelberg, Germany) as described above. The AERP and the incidence of AF were measured and calculated. Experiments were performed in unrestraint conscious dogs, therefore any effects of anesthetics (Freeman et al., 1990; Baczkó et al., 1997) on AERP and AF could be ruled out.

Action potential (AP) recordings with the conventional microelectrode technique

AP measurements from canine atrial trabeculae

Male Beagle dogs (weighing 12-15 kg; n=6) were sedated (xylazine, 1 mg/kg, i.v. and ketamine, 10 mg/kg, i.v.) and anesthetized (pentobarbital, Sigma-Aldrich, 30 mg/kg i.v.), their hearts were rapidly removed through right lateral thoracotomy. The hearts were immediately rinsed in oxygenated modified Locke's solution containing (in mM): NaCl 128.3, KCl 4, $CaCl_2$ 1.8, MgCl2 0.42, $NaHCO_3$ 21.4, and glucose 10. The pH of the solution was set between 7.35 and 7.4 when saturated with the mixture of 95% oxygen and 5% CO_2 at 37 ºC. Isolated right atrial trabeculae were obtained, individually mounted in a tissue chamber and stimulated as decribed previously (Juhász et al., 2018). The maximal rate of depolarization (V_{max}), maximum diastolic potential, action potential amplitude, and action potential duration measured at 90% of repolarization (APD_{90}) were evaluated off-line, applying stimulation with a constant basic cycle length (BCL) of 500 ms.

AP measurements from canine and rabbit right ventricular papillary muscle and in canine Purkinje fibers

Male Beagle dogs (weighing 12-15 kg; n=7) and white rabbits (weighing 2-3 kg; n=6) were used for the experiments. Right ventricular papillary muscle tips were obtained, mounted and stimulated using the conventional microelectrode technique as described previously (Jost et al., 2013; Kohajda et al., 2016). The preparations were stimulated (HSE stimulator type 215/II) initially at a constant cycle length of 500 ms (rabbit papillary muscle and canine Purkinje fibers) or 1000 ms (canine papillary muscle), with rectangular constant current pulses 2 ms in duration. The current pulses were isolated from ground and delivered through bipolar platinum electrodes. Transmembrane potentials were recorded with the use of conventional 5–20 MΩ, 3 M KCl-filled microelectrodes connected to the input of a high-impedance electrometer (Biologic Amplifier VF 102, Claix, France). The first derivative of transmembrane potential (dV/dt_{max}) was obtained electronically with a Biologic DV-140 (Claix, France) differentiator. At least 1 h was allowed for each preparation to equilibrate during continuous superfusion with modified Locke's solution, warmed to 37˚C before the experimental measurements commenced. The following types of stimulation in the course of the experiments were applied: stimulation with a constant cycle length of 1000 or 500 ms (1 or 2 Hz); stimulation with different constant cycle lengths ranging from 300 to 5000 ms taking the measurements after the 25^{th} beat. The preparations were then superfused with the solution containing 1 μM SZV-270 for 40–60 min before the pacing protocol was repeated and the parameters were measured again, then superfusion continued with 5 μM SZV-270 for another 40-60 min and measurements were repeated. Efforts were made to maintain the same impalement throughout each experiment. In case an impalement became dislodged, however, adjustment was performed and the experiment continued if AP characteristics of the re-established impalement deviated less than 5% from the previous measurement.

Whole cell patch-clamp studies

Isolated ventricular cardiomyocytes were obtained from male rabbits (weighing 2-3 kg) by enzymatic dissociation as described previously (Major et al., 2016). A drop of cell suspension was placed into a transparent recording chamber mounted on the stage of an inverted microscope (Olympus IX51, Olympus, Tokyo, Japan), and myocytes were allowed to settle and adhere to the bottom of the chamber for at least 5 minutes before superfusion was initiated. HEPES buffered Tyrode's solution was used as the normal superfusate. This solution contained (in mM): NaCl 144, NaH_2PO_4 0.4, KCl 4.0, $CaCl_2$ 1.8, $MgSO_4$ 0.53, Glucose 5.5, and HEPES 5.0 at pH of 7.4. Patch clamp micropipettes were made from borosilicate glass capillaries using a P-97 Flaming/Brown micropipette puller (Sutter Co, Novato, CA, USA). The electrodes had 1.5-2.5 MΩ resistances when filled with pipette solution that contained (in mM): KOH 110, KCl 40, K_2ATP 5, $MgCl_2$ 5, EGTA 5, GTP 0.1 and HEPES 10, during K^+ current measurements. Aspartic acid was used to adjust the pH of the pipette solution to 7.2. The L-type calcium current ($I_{Ca,L}$) was recorded in HEPES-buffered Tyrode's solution supplemented with 3 mM 4-aminopyridine. A special pipette solution was used containing (in mM: KOH 40, KCl 110, TEACl 20, MgATP 5, EGTA 10, HEPES 10 and GTP 0.25, pH was adjusted to 7.2 by KOH.

Ionic membrane currents were recorded with the Axopatch 200B patch-clamp amplifier (Molecular Devices, Sunnyvale, CA, USA) using the whole cell configuration of the patch clamp technique. Membrane currents were digitized and recorded under software control (Digidata 1440A, pClamp 10, Molecular Devices, Sunnyvale, CA, USA) after low-pass filtering at 1 kHz. The inward rectifier (I_{K1}), transient outward (I_{to}), rapid ($I_{Kr)}$ delayed rectifier potassium currents were recorded in rabbit ventricular myocytes. 1 μM nisoldipine was included in the bath solution to block $I_{Ca,L}$. When I_{Kr} was recorded, I_{Ks} was inhibited by using the selective I_{Ks} blocker HMR1556 (0.5 μM). All experiments were performed at 37 °C.

Statistical analysis

The incidence of arrhythmias was calculated and compared by using the χ^2 method. All other data are expressed as mean ± SEM. Statistical analysis was performed using ORIGIN 8.1 (Microcal Software, Northampton, MA, USA). Differences between means were compared by ANOVA followed by Student's t-test (paired or unpaired, as appropriate). Data were considered as statistically significant when $p<0.05$.

Results

Effects of SZV-270 on blood pressure and ECG parameters in anesthetized rabbits

There were no significant differences in the mean arterial blood pressures (MABP) between the control and the SZV-270 (0.3 mg/kg, i.v.) treated rabbits 5 min following SZV-270 administration (80 ± 4.9 vs. 77 ± 3.9 mmHg, respectively, $p>0.05$). Coronary artery occlusion led to a significant reduction in MABP in both groups (59 ± 5.3 vs 80 ± 4.9 mmHg in controls and 56 ± 3.8 vs 77 ± 3.9 in the SZV-270 group, all $p<0.05$, measured at 1 min following occlusion). SZV-270 administration did not change heart rate in anesthetized rabbits (251 ± 10.1 after infusion vs 258 ± 6.5 beats/min before infusion, $p>0.05$). Coronary artery occlusion and reperfusion did not change heart rate significantly in any of the groups. SZV-270 administration moderately but significantly prolonged the QTc interval in these animals (172 ± 3.3 vs 165 ± 4.4 ms before infusion, $p<0.05$). There were no significant changes in QTc intervals during reperfusion. The PQ interval did not change following SZV-270 infusion (56 ± 1.3 vs 54 ± 1.8 ms before infusion, $p>0.05$). The QRS interval was widened by SZV-270 (35 ± 1.9 ms at baseline vs 44 ± 1.8 following SZV-270 infusion, $p<0.05$).

Effects of SZV-270 on coronary artery occlusion/reperfusion induced ventricular arrhythmias in anesthetized rabbits

No arrhythmias were observed during the infusion of SZV-270 or vehicle, or following infusion preceding coronary artery occlusion. There were no differences between the control group and the SZV-270 treated group regarding the incidence of VT (40% in controls and 20% in the SZV-270 group, $p>0.05$) or VF (30% in controls and 10% in the SZV-270 group, $p>0.05$) during 10 min of coronary artery occlusion.

Arrhythmias induced by reperfusion of the occluded coronary artery occurred within 10-30 s following the ligature release. Importantly, 0.3 mg/kg SZV-270 pretreatment significantly reduced the incidence of reperfusion-induced VF (20% in SZV-270 treated group vs 80% in controls, $p<0.05$). The incidence of VT and Salvos were not decreased significantly (60% in the SZV-270 group vs 90% in controls for both arrhythmia types, all $p>0.05$).

Effects of SZV-270 in an anesthetized rabbit proarrhythmia model

Antiarrhythmic drugs that prolong repolarization can provoke Torsade de Pointes (TdP) polymorphic ventricular arrhythmias. Therefore, the proarrhythmic potency of SZV-270 was evaluated and compared to that of the pure Class III compound, dofetilide, using our previously developed anesthetized rabbit proarrhythmia model, characterized by the pharmacological reduction of repolarization reserve (Lengyel et al., 2007). In anesthetized rabbits, repolarization reserve was impaired by the i.v. administration of the selective I_{Ks} blocker HMR1556 (0.1 mg/kg) (Gögelein et al., 2000), followed by either the i.v. infusion of the selective I_{Kr} blocker dofetilide (25 μg/kg) or SZV-270 (0.3 mg/kg). A group of rabbits received dofetilide on its own to allow comparison of the effects of the I_{Kr} blocker alone to the rest of the experimental groups.

The I_{Ks} blocker HMR1556 did not change the QRS intervals (33 ± 0.9 vs 33 ± 0.6 ms at baseline, $p>0.05$) and did not influence heart rate (269 ± 5.2 vs 271 ± 9.8 beats/min at baseline, $p>0.05$). Following HMR1556 infusion, SZV-270 (0.3 mg/kg) administration significantly widened the QRS interval, similarly to the results in our rabbits in the previous set of experiments subjected to coronary artery occlusion/reperfusion (see above). (**Fig. 2A**). The combination of HMR1556 and SZV-270 did not change the PQ interval in anesthetized rabbits (63 ± 1.3 in control vs 62 ± 1.4 following HMR1556 and 62 ± 1.6 ms following HMR1556+SZV-270, all $p>0.05$). SZV-270 decreased heart rate following HMR1556 administration (230 ± 6.8 vs 269 ± 5.2 beats/min, $p<0.05$). The I_{Ks} blocker HMR1556 did not

increase the QTc interval (**Fig. 2B**). Dofetilide, alone and in combination with HMR1556, and SZV-270 in combination with HMR1556 significantly prolonged the QTc interval (**Fig. 2B**).

A novel, more reliable ECG biomarker for the prediction of drug-induced ventricular arrhythmias is short-term variability of the QT interval (STV_{QT}). STV_{QT} was moderately increased after dofetilide administration and markedly increased after the combined administration of HMR1556 and dofetilide (**Fig. 2C**). These changes were in good correlation with the increase in the incidence of TdP in these groups (**Fig. 2D**). Importantly, following impairment of repolarization reserve with the I_{Ks} blocker HMR1556, SZV-270 administration did not increase STV_{QT} (**Fig. 2C**) nor did it induce any TdP in anesthetized rabbits (**Fig. 2D**), suggesting no proarrhythmic potential of SZV-270 in this model.

Effects of SZV-270 and dofetilide on burst-induced atrial fibrillation in conscious dogs

Before starting the chronic rapid atrial pacing at 400 beats/min in conscious dogs, the right atrial effective refractory period (AERP) values in these animals were 128 ± 3.2 ms (n=6, at basic cycle length of 300 ms). The AERP significantly decreased to 88 ± 2.8 ms following 6 weeks of rapid right atrial pacing, indicating marked electrical remodeling of the right atrium. In all dogs, the effects of SZV-270 on AERP and incidence of AF were compared to that of the I_{Kr} blocker dofetilide. As **Figure 3** illustrates, both SZV-270 and dofetilide significantly prolonged AERP and markedly reduced the incidence of AF in unrestraint conscious dogs.

Effects of SZV-270 on action potentials in rabbit and canine right ventricular papillary muscle

In the following sets of *in vitro* experiments, the possible mechanisms responsible for the atrial and ventricular antiarrhythmic effects of SZV-270 were investigated. First, the effects of SZV-270 (1 and 5 μM) were studied on different action potential parameters in rabbit and canine right ventricular papillary muscle preparations using the conventional microelectrode technique. As **Table 1** and **2** illustrate, SZV-270 at 1 Hz stimulation frequency did not alter

resting membrane potential (RMP), maximum rate of depolarization (V_{max}) and action potential amplitude (APA) in rabbit and dog papillary muscle. However, SZV-270 exerted Class III antiarrhythmic effects by prolonging the action potential duration at 50%, 75% and 90% of repolarization (APD_{50}, APD_{75} and APD_{90}) and the effective refractory period in a concentration dependent manner in both species (**Fig. 4A, Fig. 5A, Table 1 and 2**). The cycle length dependent effects of SZV-270 (1 and 5 μM) were also studied in rabbit right ventricular papillary muscle preparations (**Fig. 4B** and **C**). In the higher applied concentration, SZV-270 exerted Class I/B antiarrhythmic effect in both species: it significantly decreased V_{max} at cycle lengths shorter than 1000 ms (**Fig. 4B and Fig. 5B**).

Effects of SZV-270 on action potentials in canine Purkinje fibers

SZV-270 did not alter the RMP or the APA in dog Purkinje fibers (**Table 4**). SZV-270 exerted more complex effects on action potential duration in Purkinje fibers compared to papillary muscle preparations. As shown in **(Table 4)**, at 2 Hz stimulation frequency the compound significantly prolonged the APD_{75} and APD_{90}, however, the APD prolongation was smaller following the application of the larger concentration than that observed after the application of the smaller concentration. The larger concentration significantly shortened APD_{50}. SZV-270 significantly reduced V_{max} in a concentration dependent manner (**Table 4**).

Effects of SZV-270 on action potentials in canine atrial trabeculae

In the next set of experiments, the effects of SZV-270 (1 and 5 μM) on atrial action potentials were investigated in isolated canine atrial trabeculae. SZV-270 did not change the RMP, V_{max} and APA at 2 Hz stimulation frequency in dog atrial preparations (**Table 3**). Importantly, SZV-270 significantly prolonged atrial action potentials (**Fig. 6A**), APD_{50}, APD_{75} and APD_{90} in a concentration dependent manner (**Table 3**). These effects can, at least in part, be responsible for the observed atrial antiarrhythmic effects of SZV-270 in our dog AF model.

Effects of SZV-270 on various transmembrane ionic currents in isolated rabbit ventricular myocytes

To elucidate the cellular mechanisms that can be responsible for the observed *in vivo* and *in vitro* effects of SZV-270, rabbit right ventricular myocytes were isolated and the effects of the compound were studied on I_{Kr}, I_{K1}, I_{to} and $I_{Ca,L}$ using the patch-clamp technique in the whole cell configuration. As **Figure 7** shows, SZV-270 significantly inhibited I_{Kr} in relatively low, 100 and 500 nM concentrations. This result is in agreement with the APD prolonging and QTc lengthening effect of the compound. SZV-270 did not influence the other transmembrane currents, I_{K1} (**Fig. 8A**), I_{to} (**Fig. 8B**) and $I_{Ca,L}$ (**Fig. 9**), even at the high concentration of 10 μM.

Discussion

In this study, the cardiac cellular electrophysiological and *in vivo* antiarrhythmic effects of SZV-270, a novel compound with a structure that features Class I/B and Class III structural elements (of D-sotalol and mexiletine), were investigated in dogs and rabbits, two species used frequently in arrhythmia research. Importantly, the proarrhythmic potency of SZV-270 was also assessed in a rabbit proarrhythmia model based on pharmacological impairment of repolarization reserve.

In a rabbit model of coronary artery occlusion/reperfusion, SZV-270 significantly reduced the the number of animals that died due to irreversible ventricular fibrillation during the reperfusion period. In this model, SZV-270 did not change heart rate and the PQ interval duration, however, it significantly widened QRS interval and prolonged the frequency corrected QT interval. To elucidate the mechanisms underlying the ventricular antiarrhythmic effects of SZV-270, action potential measurements were performed in rabbit and dog right ventricular papillary muscle

preparations, and several ionic currents were also measured in isolated rabbit right ventricular cardiomyocytes. In agreement with the observed QTc prolonging effect of SZV-270 (**Fig. 2B**), the compound lengthened the effective refractory period, APD_{50}, APD_{75} and APD_{90} in a concentration dependent manner in ventricular preparations in both species (**Table 1 and 2**). Furthermore, SZV-270 significantly inhibited the I_{Kr} tail current at relatively low concentrations of 100 and 500 nM (**Fig. 7**). These Class III antiarrhythmic effects were supplemented by Class I/B effects of SZV-270 in the present study. In right ventricular preparations isolated from dogs and rabbits, the larger investigated concentration of SZV-270 significantly reduced V_{max} at stimulation cycle lengths shorter than 1000 ms (**Figs. 4B and 5B**). In addition, the larger concentration of SZV-270 prolonged APD_{90} in a lesser degree and significantly shortened APD_{50} (depressed the plateau phase) in dog Purkinje fibers (**Table 4**). These effects can decrease repolarization heterogeneity in the ventricle, resembling a similar effect of amiodarone (Papp et al., 1996). Even high concentrations of SZV-270 did not affect I_{K1}, I_{to} and $I_{Ca,L}$ in rabbit right ventricular cardiomyocytes (**Figs. 8 and 9**). Indeed, the PQ interval was not altered by SZV-270 infusion in anesthetized rabbits in this study, further supporting the lack of effect of the compound on $I_{Ca,L}$.

Based on the results of this study, SZV-270 exhibits combined Class I/B and Class III antiarrhythmic actions. What makes this combination beneficial? Class III drugs prolong repolarization and the effective refractory period and are especially effective against re-entry arrhythmias (Lynch et al., 1985; Hohnloser et al., 1995; Fei and Frame, 1996). However, Class III compounds possess marked proarrhythmic activity: they promote EAD formation and subsequent development of TdP polymorphic ventricular tachycardia (Buchanan et al., 1993; Vos et al., 1995; Gottlieb et al., 1997). Drugs with ClassI/B actions, however, can reduce EAD formation (Papp et al., 1996; Sicouri et al., 1997) and have been shown to suppress TdP induced by pure Class III agents (Shimizu and Antzelevitch, 1997; Assimes and Malcolm, 1998). Also,

the combination of the Class I/B drug mexiletine and the Class III compound sotalol prevented ventricular tachyarrhythmias in dogs with myocardial infarction (Chezalviel et al., 1993). Luderitz et al. also suggested that the combination of mexiletine and sotalol was able to suppress ventricular arrhythmias more effectively than either compound alone (Luderitz et al., 1991). These results strongly suggest that a compound with combined Class I/B and III effects can prevent re-entry arrhythmias with reduced risk of provoking TdP arrhythmia.

Compounds that prolong cardiac ventricular repolarization, manifested as QT lengthening on the ECG, as mentioned above, have been associated with severe proarrhythmic adverse effects (Haverkamp et al., 2000; Redfern et al., 2003). However, QT prolongation does not necessarily cause TdP (Hondeghem et al., 2001; Milberg et al., 2002; Thomsen et al., 2004;). In this regard, several biomarkers have been proposed for improved prediction of proarrhythmic risk. Among those, the use of the short-term variability of the QT interval, characterizing the beat-to-beat variability of the QT interval and therefore the temporal variability of repolarization, has been suggested (for a review see Varkevisser et al., 2012). Indeed, both animal experimental (Thomsen et al., 2004; Lengyel et al., 2007) and clinical studies (Hinterseer et al., 2009; Hinterseer et al., 2010) have shown that the STV_{QT} was a superior biomarker for severe ventricular arrhythmia predictor compared to QT prolongation and other conventional ECG parameters. In the present study, SZV-270 exerted atrial and ventricular antiarrhythmic effects, due to, at least in part, to its I_{Kr} blocking, APD and QT prolonging properties. Therefore, we studied its proarrhythmic potency in a rabbit proarrhythmia model characterized by pharmacological impairment of repolarization reserve (Lengyel et al., 2007). The concept of repolarization reserve (Roden 1998) suggests that cardiac repolarization is redundant: inhibition or impairment of one of the repolarizing potassium currents does not lead to marked repolarization prolongation, since other currents can compensate for the lost repolarizing function (Varro et al., 2000; Roden 2006; Varro and Baczko 2011). In case, however,

repolarization reserve is attenuated by the inhibition or loss of function of I_{Ks}, a key current for repolarization reserve (Jost et al., 2005), even a mild inhibition of I_{Kr} and other repolarizing currents can lead to severe ventricular arrhythmia development (Lengyel et al., 2007; Varro and Baczko, 2001). In the present study, we found no proarrhythmic adverse effects of SZV-270. Following the impairment of repolarization reserve by the administration of the selective I_{Ks} blocker HMR1556 (Gögelein et al, 2000), SZV-270 did not increase STV_{QT} and did not induce any TdP in anesthetized rabbits. In contrast, the selective I_{Kr} blocker dofetilide significantly increased STV_{QT} and provoked TdP in 85% of rabbits following I_{Ks} inhibition (**Fig. 2D**).

In a conscious dog model of atrial fibrillation that is based on chronic right atrial tachypacing-induced atrial electrical remodeling (Morillo et al., 1995), SZV-270 significantly reduced the incidence of burst-induced AF and prolonged the AERP (**Fig. 3**). In canine right atrial trabeculae, SZV-270 prolonged the APD_{50}, APD_{75} and APD_{90} in a concentration dependent manner (**Table 3**). The effects of SZV-270 on AF in this model were comparable to those of the selective I_{Kr} blocker dofetilide, which is known as an effective compound for rhythm control in AF (Kirchhof 2016; Piccini and Fauchier 2016). Dofetilide also reduced AF incidence and increased AERP in the present study, and was shown to prolong atrial APD in atrial trabeculae isolated from dogs with chronic tachypacing induced atrial remodeling (Juhász et al., 2018). The beneficial effects of dofetilide in AF were attributed to its atrial repolarization and AERP prolonging effects (Allessie et al., 2001; Pedersen et al., 2001). The AF incidence reducing effects of SZV-270 is also probably due to its atrial APD prolonging effects in this study.

Conclusions

In conclusion, SZV-270 protected against coronary artery occlusion/reperfusion-induced ventricular arrhythmias in rabbits. SZV-270 significantly reduced the incidence of atrial fibrillation and prolonged atrial effective refractory period in a conscious dog model of atrial

fibrillation. Our cellular electrophysiological investigations revealed that SZV-270 exerted its ventricular and atrial antiarrhythmic effects via combined Class I/B and Class III actions. Importantly, despite its I_{Kr} blocking and QT prolonging properties, SZV-270 did not provoke TdP arrhythmia in an anesthetized rabbit proarrhythmia model.

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Parameter	Control	SZV-270 1 µM	SZV-270 5 µM
RMP (mV)	-86.3 ± 1.8	-84.2 ± 1.2	-85.2 ± 1.4
APA (mV)	115.7 ± 1.8	112.8 ± 3.3	113.5 ± 2.5
APD_{10} (ms)	54.8 ± 7.2	49.6 ± 7.4	50.8 ± 9.1
APD_{25} (ms)	105.2 ± 11.5	108.2 ± 14.6	114.5 ±16.2
APD_{50} (ms)	152.3 ± 15.4	178.7 ± 18.9 *	221.2 ± 23.9*
APD_{75} (ms)	174.3 ± 15.6	209.0 ± 18.6 *	263.7 ± 23.9*
APD_{90} (ms)	183.3 ±15.4	219.3 ± 18.5 *	274.5 ± 23.7*
V_{max} (V/s)	229.8 ± 24.3	231.2 ± 23.2	241.0 ± 22.1
ERP (ms)	174.7 ± 14.7	223.8 ± 20.4 *	277.5 ± 27.5*

Table 1. Effect of SZV-270 (1 and 5 µM) on the action potential in rabbit right ventricular papillary muscle preparations (n=6). Stimulation frequency = 1 Hz; RMP = resting membrane potential; APA = action potential amplitude; $APD_{10–25–50–75–90}$ = action potential duration at 10, 25, 50, 75 and 90% of repolarisation; V_{max} = maximal rate of depolarization; ERP = effective refractory period. Results are expressed as means ± SEM; *p<0.05.

476

Parameter	Control	SZV-270 1 µM	SZV-270 5 µM
RMP (mV)	-84.9 ± 1.0	-85.4 ± 1.0	-83.9 ± 1.1
APA (mV)	105.4 ± 1.3	107.3 ± 1.3*	106.0 ± 2.4
APD_{10} (ms)	60.9 ± 13.3	66.3 ± 14.2	58.9 ±16.7
APD_{25} (ms)	133.0 ± 6.9	143.9 ± 8.0*	149.4 ± 7.7
APD_{50} (ms)	180.0 ± 6.8	198.3 ± 9.9*	208.0 ±11.4
APD_{75} (ms)	201.6 ± 7.5	222.6 ± 11.0*	240.7 ± 10.4*
APD_{90} (ms)	211.4 ± 7.9	233.6 ± 11.2*	251.7 ± 10.6*
V_{max} (V/s)	208.0 ± 8.9	220.6 ± 13.0	212.0 ± 12.7
ERP (ms)	223.4 ± 8.3	250.0 ± 14.1*	263.4 ± 13.1*

477

478 **Table 2.** Effect of SZV-270 (1 and 5 µM) on the action potential in canine right ventricular
479 papillary muscle preparations (n=7). Stimulation frequency = 1 Hz; RMP = resting membrane
480 potential; APA = action potential amplitude; $APD_{10–25–50–75–90}$ = action potential duration at 10,
481 25, 50, 75 and 90% of repolarisation; V_{max} = maximal rate of depolarization; ERP = effective
482 refractory period. Results are expressed as means ± SEM; *p<0.05.

483

484

Parameter	Control	SZV-270 1 µM	SZV-270 5 µM
RMP (mV)	-85.7 ± 1.2	-85.2 ± 1.6	-85.5 ± 1.1
APA (mV)	109.0 ± 1.0	109.3 ± 1.6	111.5 ± 2.5
APD_{10} (ms)	9.0 ± 0.7	9.2 ± 0.5	9.5 ± 0.8
APD_{25} (ms)	43.8 ± 5.1	46.6 ± 4.6	47.4 ± 4.2
APD_{50} (ms)	74.0 ± 5.8	81.8 ± 8.0*	83.8 ± 6.6*
APD_{75} (ms)	100.2 ± 4.8	115.0 ± 8.8*	120.5 ± 8.0*
APD_{90} (ms)	130.8 ± 4.1	156.0 ± 9.6*	165.0 ± 9.4*
V_{max} (V/s)	299.8 ± 38.8	343.0 ± 37.8	347.2 ± 45.8

Table 3. Effect of SZV-270 (1 and 5 µM) on the action potential in canine atrial trabecular preparations (n=6). Stimulation frequency = 2 Hz; RMP = resting membrane potential; APA = action potential amplitude; $APD_{10–25–50–75–90}$ = action potential duration at 10, 25, 50, 75 and 90% of repolarisation; V_{max} = maximal rate of depolarization. Results are expressed as means ± SEM; *p<0.05.

Parameter	Control	SZV-270 1 µM	SZV-270 5 µM
RMP (mV)	-89.3 ± 0.8	-90.2 ± 0.6	-89.2 ± 0.6
APA (mV)	125.0 ± 1.9	127.2 ±1.5	123.7 ± 1.5
APD_{10} (ms)	1.77 ± 0.15	1.77 ± 0.17	1.73 ± 0.21
APD_{25} (ms)	32.6 ± 9.6	30.5 ± 10.0	24.6 ± 7.2
APD_{50} (ms)	174.7 ±11.1	186.8 ± 13.9	144.2 ± 12.1*
APD_{75} (ms)	229.3 ± 6.3	271.5 ± 9.6*	245.5 ± 7.1*
APD_{90} (ms)	250.0 ± 6.3	301.0 ± 10.2*	285.0 ± 9.1*
V_{max} (V/s)	730.8 ± 67.6	704.7 ± 64.3*	684.8 ± 43.4*

Table 4. Effect of SZV-270 (1 and 5 µM) on the action potential in canine Purkinje fibers (n=6). Stimulation frequency = 2 Hz; RMP = resting membrane potential; APA = action potential amplitude; $APD_{10–25–50–75–90}$ = action potential duration at 10, 25, 50, 75 and 90% of repolarisation; V_{max} = maximal rate of depolarization; *$p<0.05$

Figure legends

Fig. 1. Chemical structure of SZV-270.

Fig. 2. The effects of the I_{Ks} blocker HMR1556 (0.1 mg/kg, i.v.), the I_{Kr} blocker dofetilide (25 μg/kg, i.v.) and SZV-270 (0.3 mg/kg, i.v.) on different ECG parameters and the incidence of Torsades de Pointes (TdP) arrhythmia in an anesthetized rabbit proarrhythmia model. (**A**) Only SZV-270 widened the QRS interval, while (**B**) the frequency corrected QT interval (QTc) was prolonged by dofetilide, the combination of HMR1556+dofetilide and HMR1556+SZV270. (**C**) Despite prolonging QTc, the combination of HMR1556+SZV270 did not increase the short-term variability of the QT interval (STV_{QT}), a surrogate biomarker for the prediction of ventricular arrhythmias. (**D**) In parallel with a markedly and significantly increased STV_{QT}, only the combination of HMR1556+dofetilide led to a high incidence of TdP. SZV-270 did not show any proarrhythmic activity in this model with impaired repolarization reserve. Values are mean ± SEM. #$p<0.05$ vs. baseline values within the same group; *$p<0.05$ vs. dofetilide group; n=8-11 rabbits/group.

Fig. 3. Effect of the selective I_{Kr} blocker dofetilide (25 μg/kg, i.v.) and SZV-270 (0.3 mg/kg, i.v.) on atrial fibrillation in conscious dogs with atrial tachypacing-induced electrical atrial remodeling. (**A**) Both dofetilide and SZV-270 significantly increased right atrial effective refractory period (AERP). (**B**) Both dofetilide and SZV-270 significantly reduced the incidence of atrial fibrillation (AF). AERP was measured at basic cycle length of 300 ms. Values are mean ± SEM; n=4-6 animals/group; *$p<0.05$ vs control values.

Fig. 4. Effect of SZV-270 (1 and 5 μM) on the action potential, on V_{max} and APD_{90} at different stimulation cycle lengths recorded from rabbit right ventricular papillary muscle preparations. (**A**) SZV-270 prolonged the action potential in rabbit right ventricular papillary muscle. (**B**) SZV-270 (5 μM) significantly reduced V_{max} at 300 ms cycle length, (**C**) and both concentrations significantly prolonged APD_{90} at cycle lengths shorter than 3000 ms in these preparations. Values are means ± SEM. n=6, *$p<0.05$ vs. control values.

Fig. 5. Effect of SZV-270 (1 and 5 μM) on the action potential, on V_{max} and APD_{90} at different stimulation cycle lengths recorded from dog right ventricular papillary muscle preparations. (**A**) SZV-270 prolonged the action potential in canine right ventricular papillary muscle. (**B**) SZV-270 (5 μM) significantly reduced V_{max} at 300 ms cycle length, (**C**) and both concentrations significantly prolonged APD_{90} in these preparations. Values are means ± SEM. n=6, *p<0.05 vs. control values.

Fig. 6. The effects of SZV-270 (1 and 5 μM) on the action potential, on V_{max} and APD_{90} at different stimulation cycle lengths recorded from isolated canine right atrial trabeculae. (**A**) SZV-270 prolonged the action potential in dog atrial trabeculae. (**B**) SZV-270 did not significantly alter V_{max}, however, (**C**) significantly prolonged APD_{90} in these preparations. Values are means ± SEM. n=6, *p<0.05 vs. control values.

Fig. 7. The effect of SZV-270 on the rapid component of the delayed rectifier potassium current (I_{Kr}). SZV-270 inhibited the I_{Kr} tail current in a concentration dependent manner (panel **A**: effects of 100 nM, panel **B**: effects of 500 nM SZV-270). Left subpanels show original current traces in control conditions and following application of 100 and 500 nM SZV-270. Graphs on the right show the respective current-voltage relationships. Values are means ± SEM. n=3-5, *p<0.05 vs corresponding data point in control conditions.

Fig. 8. SZV-270 did not influence (**A**) I_{K1} or (**B**) I_{to} even at the high concentration of 10 μM in isolated rabbit right ventricular cardiomyocytes. Left panels depict original current traces recorded in control conditions and in the presence of 10 μM SZV-270. Right panels show the current-voltage relationships. Values are means ± SEM. n=5-6, all p>0.05.

Fig. 9. SZV-270 did not influence $I_{Ca,L}$ even at the high concentration of 10 μM in isolated rabbit right ventricular cardiomyocytes. Left panels depict original current traces recorded in control conditions, in the presence of 10 μM SZV-270 and following washout. Right panel shows the current-voltage relationship. Values are means ± SEM. n=4, all p>0.05.

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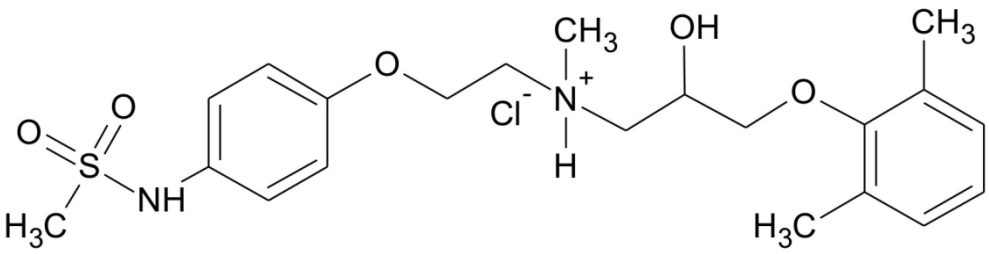

Fig. 1. Chemical structure of SZV-270.

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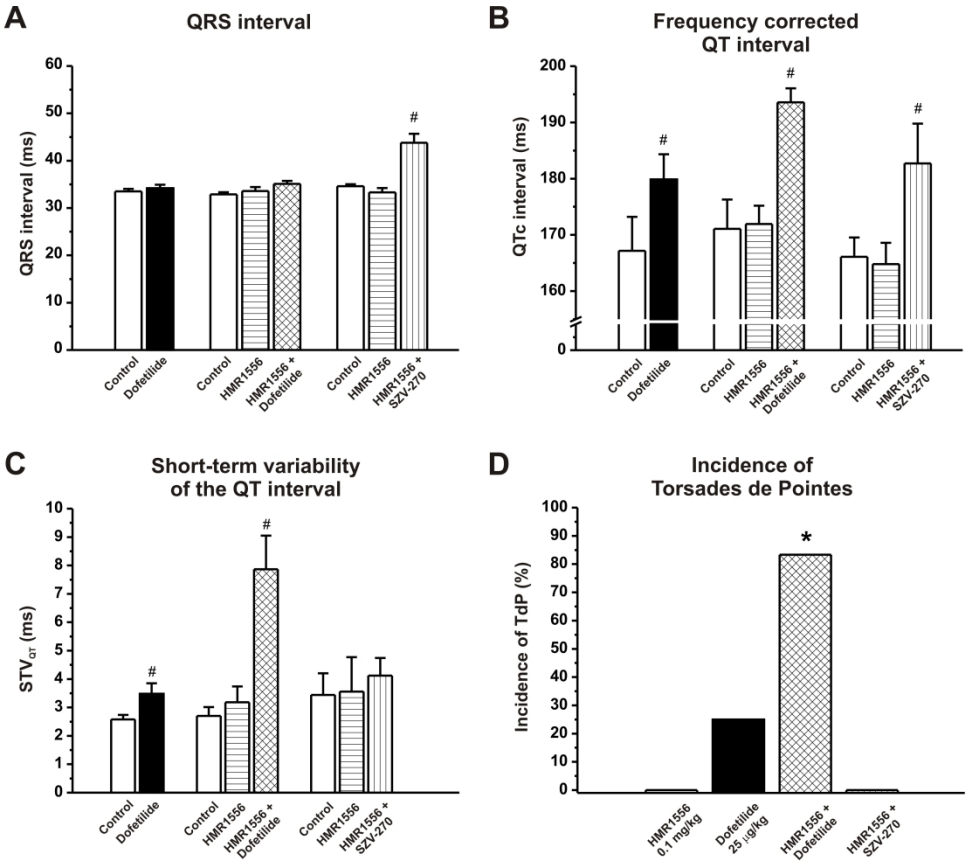

Fig. 2. The effects of the IKs blocker HMR1556 (0.1 mg/kg, i.v.), the IKr blocker dofetilide (25 µg/kg, i.v.) and SZV-270 (0.3 mg/kg, i.v.) on different ECG parameters and the incidence of Torsades de Pointes (TdP) arrhythmia in an anesthetized rabbit proarrhythmia model. (A) Only SZV-270 widened the QRS interval, while (B) the frequency corrected QT interval (QTc) was prolonged by dofetilide, the combination of HMR1556+dofetilide and HMR1556+SZV270. (C) Despite prolonging QTc, the combination of HMR1556+SZV270 did not increase the short-term variability of the QT interval (STVQT), a surrogate biomarker for the prediction of ventricular arrhythmias. (D) In parallel with a markedly and significantly increased STVQT, only the combination of HMR1556+dofetilide led to a high incidence of TdP. SZV-270 did not show any proarrhythmic activity in this model with impaired repolarization reserve. Values are mean ± SEM. #p<0.05 vs. baseline values within the same group; *p<0.05 vs. dofetilide group; n=8-11 rabbits/group.

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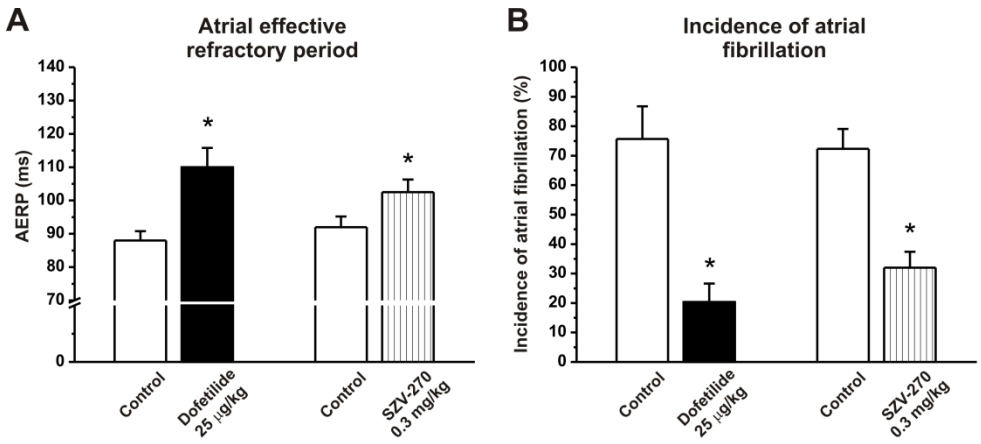

Fig. 3. Effect of the selective IKr blocker dofetilide (25 μg/kg, i.v.) and SZV-270 (0.3 mg/kg, i.v.) on atrial fibrillation in conscious dogs with atrial tachypacing-induced electrical atrial remodeling. (A) Both dofetilide and SZV-270 significantly increased right atrial effective refractory period (AERP). (B) Both dofetilide and SZV-270 significantly reduced the incidence of atrial fibrillation (AF). AERP was measured at basic cycle length of 300 ms. Values are mean ± SEM; n=4-6 animals/group; *p<0.05 vs control values.

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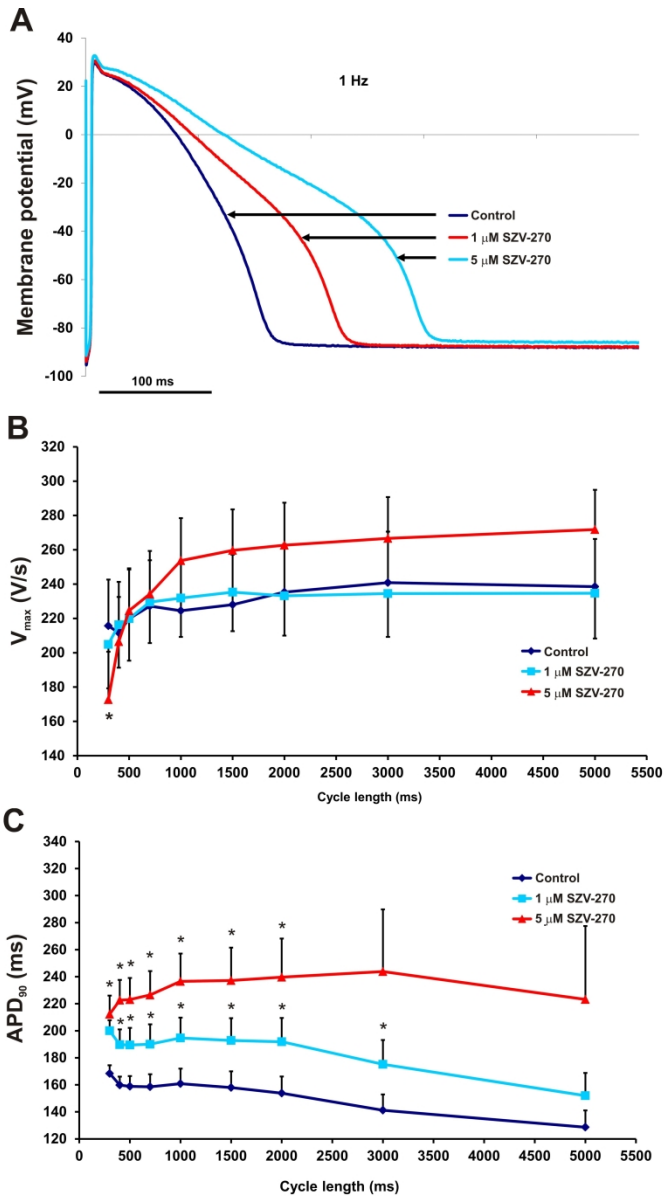

Fig. 4. Effect of SZV-270 (1 and 5 μM) on the action potential, on V_{max} and APD_{90} at different stimulation cycle lengths recorded from rabbit right ventricular papillary muscle preparations. (A) SZV-270 prolonged the action potential in rabbit right ventricular papillary muscle. (B) SZV-270 (5 μM) significantly reduced V_{max} at 300 ms cycle length, (C) and both concentrations significantly prolonged APD_{90} at cycle lengths shorter than 3000 ms in these preparations. Values are means ± SEM. n=6, *p<0.05 vs. control values.

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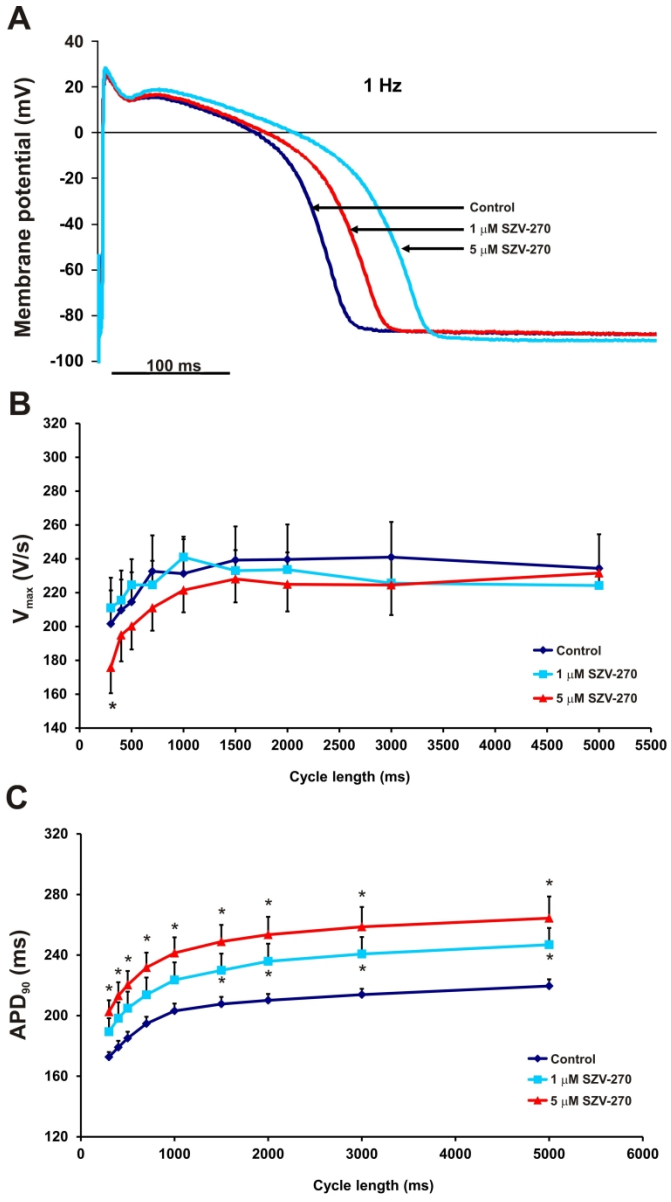

Fig. 5. Effect of SZV-270 (1 and 5 μM) on the action potential, on V_{max} and APD_{90} at different stimulation cycle lengths recorded from dog right ventricular papillary muscle preparations. (A) SZV-270 prolonged the action potential in canine right ventricular papillary muscle. (B) SZV-270 (5 μM) significantly reduced V_{max} at 300 ms cycle length, (C) and both concentrations significantly prolonged APD_{90} in these preparations. Values are means ± SEM. n=6, *p<0.05 vs. control values.

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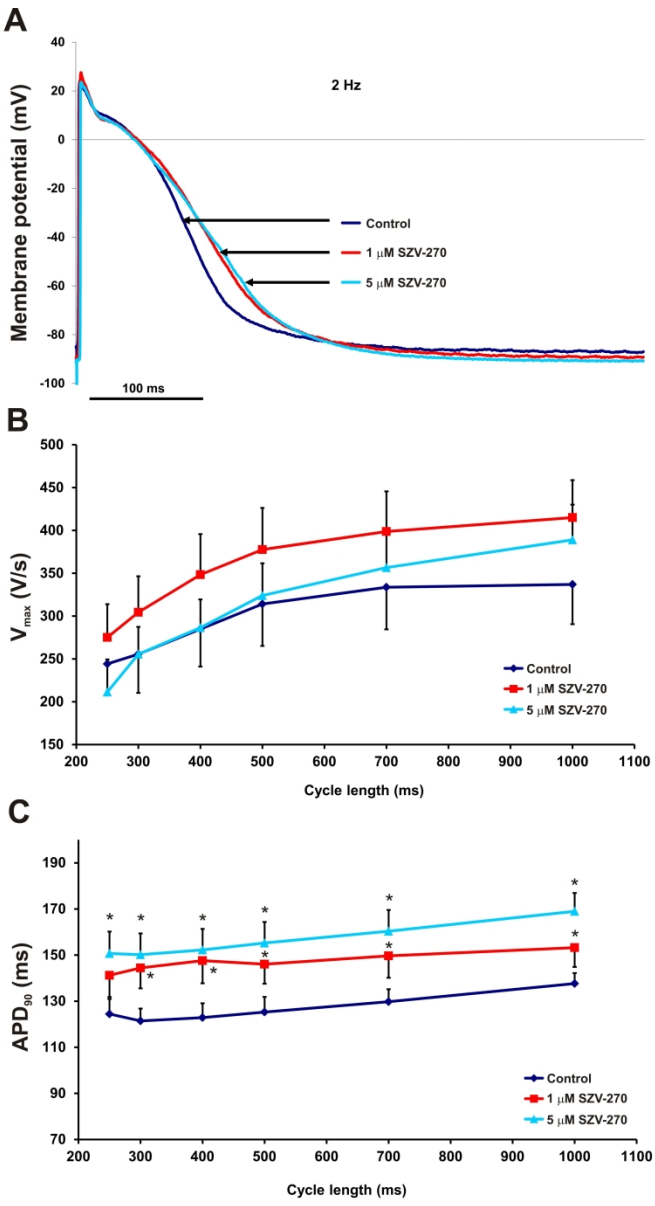

Fig. 6. The effects of SZV-270 (1 and 5 μM) on the action potential, on V_{max} and APD_{90} at different stimulation cycle lengths recorded from isolated canine right atrial trabeculae. (A) SZV-270 prolonged the action potential in dog atrial trabeculae. (B) SZV-270 did not significantly alter V_{max}, however, (C) significantly prolonged APD_{90} in these preparations. Values are means ± SEM. n=6, *p<0.05 vs. control values.

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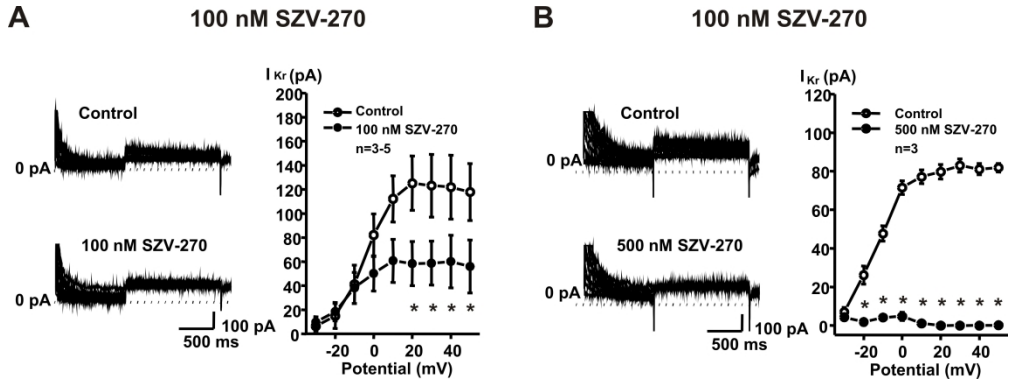

Fig. 7. The effect of SZV-270 on the rapid component of the delayed rectifier potassium current (I_{Kr}). SZV-270 inhibited the I_{Kr} tail current in a concentration dependent manner (panel A: effects of 100 nM, panel B: effects of 500 nM SZV-270). Left subpanels show original current traces in control conditions and following application of 100 and 500 nM SZV-270. Graphs on the right show the respective current-voltage relationships. Values are means ± SEM. n=3-5, *p<0.05 vs corresponding data point in control conditions.

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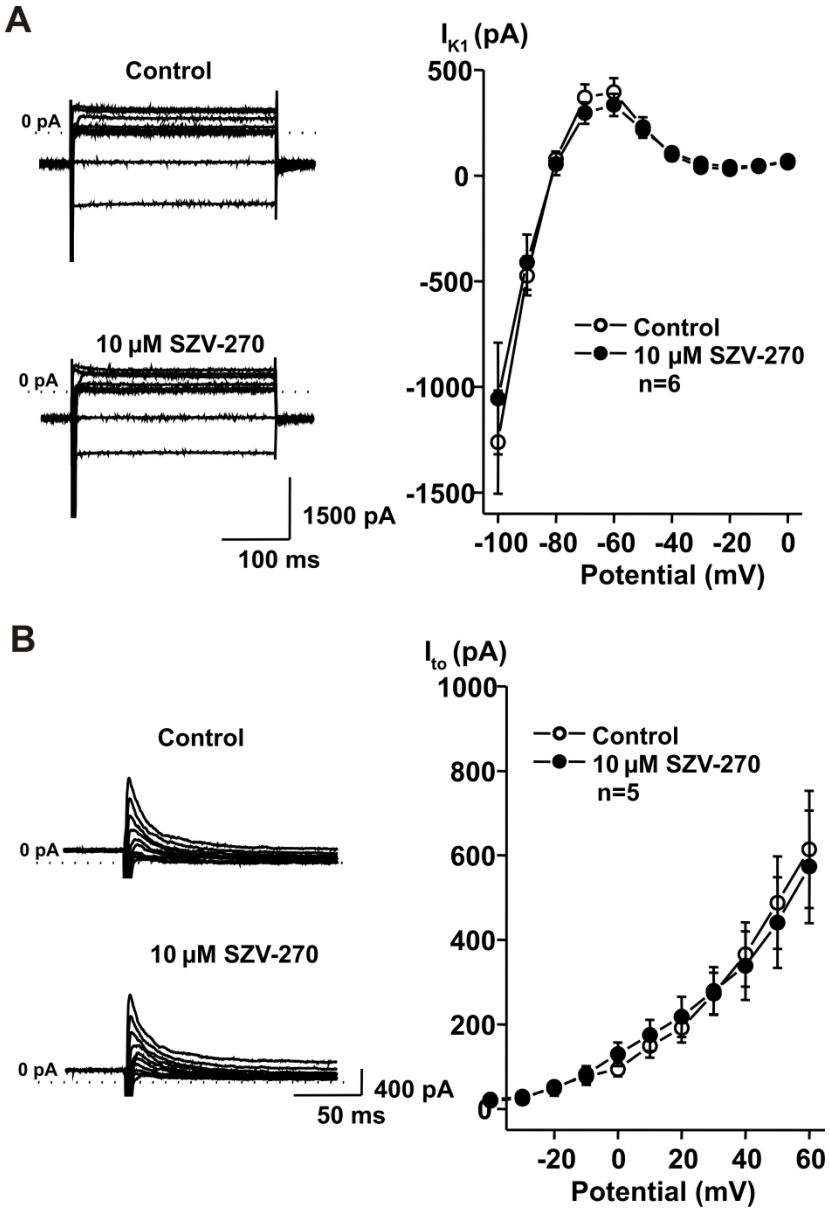

Fig. 8. SZV-270 did not influence (A) I_{K1} or (B) I_{to} even at the high concentration of 10 µM in isolated rabbit right ventricular cardiomyocytes. Left panels depict original current traces recorded in control conditions and in the presence of 10 µM SZV-270. Right panels show the current-voltage relationships. Values are means ± SEM. n=5-6, all $p>0.05$.

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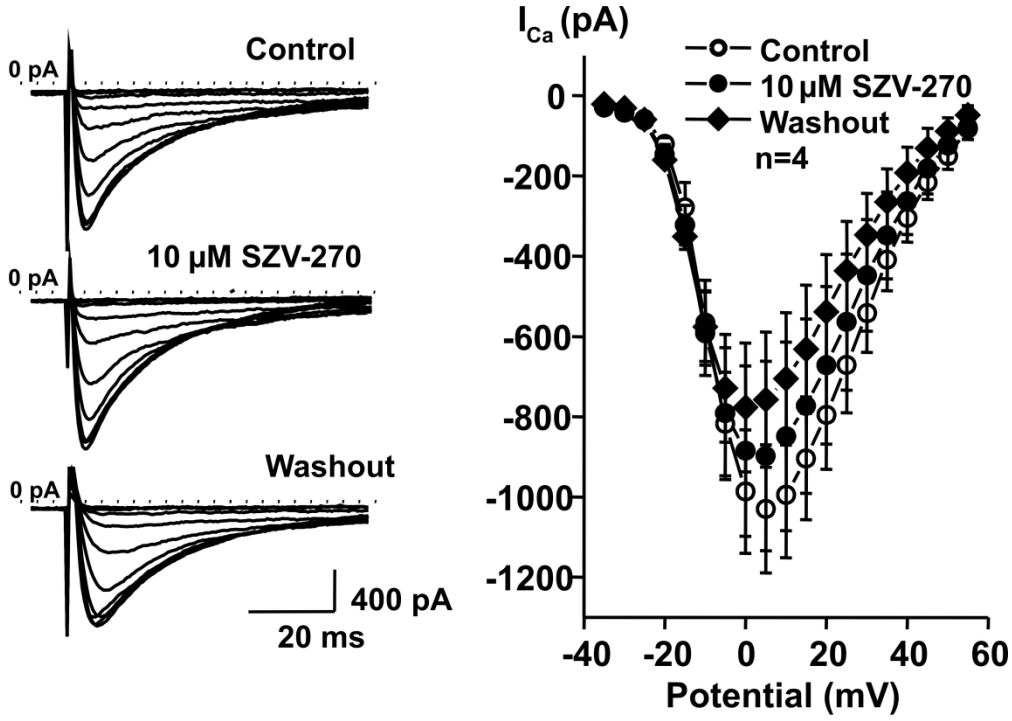

Fig. 9. SZV-270 did not influence $I_{Ca,L}$ even at the high concentration of 10 µM in isolated rabbit right ventricular cardiomyocytes. Left panels depict original current traces recorded in control conditions, in the presence of 10 µM SZV-270 and following washout. Right panel shows the current-voltage relationship. Values are means ± SEM. n=4, all $p>0.05$.

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