RSD1235 blocks late $I_{Na}$ and suppresses early afterdepolarizations and torsades de pointes induced by class III agents

Peter M.R. Orth $^{a}$, J. Christian Hesketh $^{a}$, Carmen K.H. Mak $^{a}$, Yi Yang $^{a}$, Shunping Lin $^{a}$, Gregory N. Beatch $^{a}$, Alan M. Ezrin $^{a}$, David Fedida $^{b,*}$

$^{a}$ Cardiome Pharma Corporation, 6th Floor, 6190 Agronomy Road, Vancouver BC, Canada V6T 1Z3
$^{b}$ Department of Cellular and Physiological Sciences, University of British Columbia, 2146 Health Sciences Mall, Vancouver BC, Canada V6T 1Z3

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Abstract

Objective: RSD1235 is a novel antiarrhythmic drug with atria-selective electrophysiological actions on Na$^{+}$ and K$^{+}$ currents. The mechanism for its protection of ventricular repolarization was assessed by its action on Purkinje fibers, and by block of late sodium current active during repolarization. Further, RSD1235’s ability to reverse the pro-arrhythmic actions of the class III agents dofetilide and clofilium was assessed in isolated Purkinje fibers and an in vivo model of torsades de pointes (TdP).

Methods: Action potential and early afterdepolarization (EAD) recordings were made from in situ and isolated rabbit Purkinje fibers at 37 °C using floating sharp microelectrodes; late $I_{Na}$ was recorded using a whole-cell patch clamp technique of Nav1.5 expressed in HEK cells at 22 °C; In vivo, anesthetized methoxamine-sensitized rabbits were used to test the ability of RSD1235 to suppress clofilium-induced TdP.

Results: RSD1235 (0.5–30 μM) had minor dose-dependent effects on action potential duration (APD) at 50% and 90% repolarization in Purkinje fibers, but pre-treatment significantly attenuated the APD-prolonging effects of dofetilide (300 nM). EADs induced by 300 nM dofetilide were terminated by 30 μM RSD1235 in all experiments (n = 7). RSD1235 blocked a late component of Na current ($I_{Na}$), which can produce inward currents contributing to EAD formation. RSD1235 pre-treatment (1 μmol/kg/min) or acute infusions prevented/terminated TdP induced by clofilium in 8 of 9 rabbits, and reduced the duration of TdP episodes from 71±23 s in control to 17±7 and 14±14 s at infusion rates of 0.3 and 1.0 μmol/kg/min, respectively (n = 9, p<0.001).

Conclusion: RSD1235 itself has minor actions on repolarization in Purkinje fibers, but can reverse the AP-prolonging actions of class III agents and terminate arrhythmias in a model of TdP. We suggest that these protective actions of RSD1235 may result, at least in part, from its ability to inhibit late $I_{Na}$ during action potential repolarization.

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Keywords: Antiarrhythmic drug; Torsades de pointes; Atrial fibrillation; Potassium channel block; Sodium channel block

1. Introduction

RSD1235 is a new antiarrhythmic drug currently in late-stage clinical trials that, unlike agents presently used, shows atria-selective actions and only minimally affects ventricular repolarization [1]. Unlike class III agents, RSD1235 terminates AF by causing a mixed block of cardiac K$^{+}$ and Na$^{+}$ channels [2]. The transient outward and ultra-rapid delayed rectifier currents are blocked with IC$_{50}$’s in the low micromolar range (10–30 μM) while the cardiac Na$^{+}$ channel, Nav1.5, is blocked in a rate and voltage-dependent manner with an IC$_{50}$ of ~30 μM (1 Hz, pulse from −100 to −30 mV), and the L-type Ca$^{2+}$ current is unaffected [3]. At fibrillating heart rates and depolarized potentials, the Na$^{+}$ channel potency is greatly increased, with a value of 9 μM at 20 Hz and −60 mV. This means that conduction in the
normally polarized ventricle will not be much affected. In humans, ventricular repolarization time (as measured by QT interval) and ventricular conduction velocity (QRS width) are only minimally affected by RSD1235 at peak plasma levels of 6 μg/ml (~15 μM [4]).

In the present study, we sought to further understand the lack of effect of RSD1235 on ventricular tissue by examining its actions in models of ventricular proarrhythmia caused by drugs that prolong repolarization. Currently used selective class III antiarrhythmic drugs terminate atrial fibrillation (AF) by prolonging repolarization of cardiac tissue and enhancing the refractory period [5,6]. Complications can arise because prolonging repolarization can induce oscillations in ventricular membrane potential, called early afterdepolarizations (EADs), or increase tissue dispersion of repolarization [7–10]. Both of these mechanisms can trigger a polymorphic ventricular tachycardia, also known as torsades de pointes (TdP), that can easily degenerate into ventricular fibrillation [11–13].

We found that RSD1235 had small effects on Purkinje fiber action potential duration alone, but prevented and/or reversed the action potential prolongation induced by the selective class III drug, dofetilide in rabbit Purkinje fibers. Rather than inducing TdP, RSD1235 both prevented and terminated TdP induced by clofilium in the methoxamine rabbit model. RSD1235 was an effective blocker of the late sodium current ($I_{Na}$) reactivated during repolarization, comparable to lidocaine, an agent known to block late $I_{Na}$ [14] and we suggest that this may underlie its ability to reverse the destabilization of ventricular repolarization induced by class III agents.

Fig. 1. RSD1235 attenuates the action potential prolonging effects of dofetilide. A. Purkinje fiber action potential duration (APD$_{50}$ and APD$_{90}$) recorded from attached preparations (see Methods) in dofetilide (3–300 nM) or RSD1235 (0.3–30 μM). APD$_{50}$ data are open symbols and APD$_{90}$ data are closed symbols (n=4). B. APD$_{50}$ recorded when Purkinje fiber were co-treated with 10–300 nM dofetilide and either vehicle (triangles) or 30 μM RSD1235 (circles). The identity of solutions was blinded to the experimenter (n=10). C. Left bar triplet: APD$_{50}$ in control, 30 μM RSD1235, and after addition of 300 nM dofetilide to perfusate containing 30 μM RSD1235 (n=4). Right bar triplet: APD$_{50}$ in control, 300 nM dofetilide, and after addition of 30 μM RSD1235 to perfusate containing 30 μM dofetilide (n=4). D. Left bar triplet: APD$_{90}$ in control, 300 nM dofetilide, and after addition of 30 μM RSD1235 to perfusate containing 300 nM dofetilide (n=4). Right bar triplet: APD$_{90}$ control, 300 nM dofetilide, and after addition of 30 μM RSD1235 to perfusate containing 300 nM dofetilide (n=4). E. Left bar triplet: Effective refractory period (ERP) in control, 30 μM RSD1235, and after addition of 30 μM RSD1235 to perfusate containing RSD1235 (n=4). Right bar triplet: ERP in control, 300 nM dofetilide and after addition of 30 μM RSD1235 to perfusate (n=4). Purkinje fibers were stimulated at 1 Hz in all cases. The S1–S1 interval was 1 s and 8 S1 pulses preceded the S2 pulse. Data are mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.005 relative to pre-drug values, except where indicated.
2. Methods

2.1. Rabbit Purkinje fibers

Female NZW rabbits were anesthetized with sodium pentobarbital (60 mg/kg) and then euthanized by cervical dislocation. Hearts were rapidly excised and spread open along the left-ventricular side of the interventricular septum. Action potentials (APs) were recorded using floating sharp microelectrodes (Fig. 1). In EAD experiments and some AP duration experiments (Figs. 1, 2), Purkinje fibers were isolated from the left ventricle by separating small pieces of ventricular tissue at the junctions of either side of the Purkinje fibers and then transferred to the 37 °C bath (Warner automatic temperature controller TC-324B). The ventricular tissue was pinned and the fiber gently stretched to provide an adequate surface for impalement with a floating microelectrode. In all Purkinje fiber experiments stimulation was carried out via local bipolar stimulation to one end of the fiber under study.

2.2. Rabbit torsades de pointes model

The anesthetized rabbit model was used as described by Carlsson et al., with minor modifications (see Supplemental Material) [15,16]. Female New Zealand white rabbits (2–3.5 kg) were anesthetized with 60 mg/kg sodium pentobarbital, i.v. [15,17] and additional doses were administered during surgery as necessary to maintain the depth of anesthesia (determined by eyelid reflex). All animal procedures conform with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH).

2.3. Patch clamp of Nav1.5 in HEK cells

Nav1.5 channels were stably expressed in human embryonic kidney (HEK) cells and superfused at 25 °C in a 0.5 mL bath with an external solution that contained (mM): NaCl 130; dextrose 10; HEPES 10; MgCl₂ 1; CsCl 5; CaCl₂ 1 (pH adjusted to 7.4 with NaOH). Whole-cell current recordings and analysis were made using an Axopatch 200B amplifier.

Fig. 2. RSD1235 terminates EADs induced by dofetilide. Action potentials were elicited in Purkinje fibers isolated from rabbit left ventricle. A. An example of APs recorded pre-drug at basic cycle lengths (BCLs) of 1 and 3 s. B. APs from the same impalement recorded in the presence of 300 nM dofetilide (at 8 s BCL). Note the induction of early afterdepolarizations (EADs). C. APs from the same impalement recorded after 30 μM RSD1235 was added to perfusate containing 300 nM dofetilide (BCL=3 s). Termination of all EADs required 16.8 min of RSD1235 perfusion. Similar results were obtained in 7 experiments.
and pClamp8 software (Axon Instruments, CA). Patch electrodes were pulled from thin-walled borosilicate glass (World Precision Instruments, FL) on a horizontal micropipette puller (Sutter Instruments, CA). Electrodes had resistances of 1.5–3.5 MΩ when filled with control filling solution, in mM: CsCl 130; Na2ATP 4; MgCl2 1; HEPES 5; EGTA 10 (pH adjusted to 7.2 with NaOH). Analog capacity compensation and 75–85% series resistance compensation were used in all whole cell measurements. Membrane potentials were not corrected for junction potentials that arise between the pipette and bath solution. Data were sampled at 10–20 kHz and filtered at 5 to 10 kHz. A step/ramp protocol (−100 mV step to 20 mV for 100 ms, then ramp back to −100 mV over 100 ms) was used to examine the three main components of Na+ current active during an action potential.

2.4. Detailed experimental protocols

Additional information to that in the text and figure legends may be found in the Supplemental Material.

2.5. Drugs

RSD1235 is 3-Pyrrolidinol, 1-{[1R,2R]-2-[2-(3,4-dimethoxyphenyl)ethoxy]cyclohexyl]-hydrochloride, (3R). It was synthesized at Cardiome Pharma Corp. and infused in isotonic solution for in vivo experiments. For in vitro experiments, RSD1235 was dissolved in DMSO or ultrapure H2O. No difference between data collected with the different vehicles was noted.

2.6. Statistics

ANOVA was used for analyses of rabbit Purkinje fiber APD. The Na+ current components recorded from HEK cells were analyzed using a paired r-test. The incidence of TdP was analyzed using Fisher’s exact test and changes in QTc were analyzed using an analysis of covariance model. The Wilcoxon rank sum test was used for the analysis of the duration of TdP episodes and the Product limit method and log rank test were used to analyze the time to PVC. All probability values were calculated using two-sided tests and a value of <0.05 was considered statistically significant. Unless stated otherwise, data are presented as mean±SEM.

3. Results

3.1. Effects of RSD1235 and dofetilide on Purkinje fiber APD

Rabbit Purkinje fibers are very sensitive to drugs that prolong repolarization of human ventricular tissue [18] due to the importance of rapidly activating delayed rectifier (Ikr) currents in the termination of the action potential plateau. Furthermore, class III antiarrhythmics that are proarrhythmic prolong APs in Purkinje fibers to a greater extent than in ventricular muscle [19–21]. In whole-heart rabbit Purkinje fiber tissue, we compared RSD1235 and the class III drug dofetilide. In the presence of escalating doses of dofetilide (3–300 nM), action potential duration (APD) at 1 Hz at 50% and 90% repolarization (APD50 and APD90) increased from pre-drug levels by 86±13% and 107±9% (p<0.01 and p<0.005, respectively) in the presence of 300 nM dofetilide (Fig. 1A). By contrast, doses of RSD1235 up to 30 µM increased APD50 and APD90 by only 4±4% and 13±3% (p<0.01). In further experiments the action of 3, 10 and 30 µM RSD1235, and 300 nM E4031 was examined on APD90 in isolated Purkinje fibers excised (as we described for the EAD experiments below) from the left and right ventricles of rabbit hearts. The mean APD90 at 1 Hz after the equilibration period was 310±30 ms, n=9 preparations, and the mean change in APD90 at 3, 10 and 30 µM RSD1235 was 4.3±1.7%, 15.4±2.3%, and 27.5±3.9%, respectively; p<0.05 at 10 and 30 µM compared with the pre-control APD, using one-way ANOVA. 300 nM E4031 caused a failure of repolarization in all fibers examined. These changes in isolated fibers are quite comparable with those we observed using the whole-heart fibers, but suggest an increased sensitivity when fibers are removed from the heart.

3.2. Effects of RSD1235 and dofetilide co-treatments on APD and ERP

We next tested whether co-administration of RSD1235 with dofetilide could attenuate the AP prolonging effects of dofetilide observed in Purkinje fibers. In blinded experiments, rabbit Purkinje fiber APs were recorded in dofetilide alone or dofetilide together with RSD1235. Treatment with 30 µM RSD1235 and escalating concentrations of dofetilide significantly reduced the APD50 prolongation compared to dofetilide alone (Fig. 1B). The extent to which APD50 was attenuated in the presence of RSD1235+dofetilide with respect to dofetilide alone depended on the sequence of drug addition. If 30 µM RSD1235 was added first, the effect of dofetilide addition upon APD50 or APD90 was smaller than when 300 nM dofetilide was added before 30 µM RSD1235 (Fig. 1C, D). In both cases, perfusate containing RSD1235 and dofetilide significantly increased APD50 and APD90 over pre-drug levels, but the effect was far greater when dofetilide was added first. Although dofetilide and RSD1235 reduced the APD50 prolongation induced by dofetilide alone (Fig. 1B), addition of RSD1235 to dofetilide did not significantly affect effective refractory period (ERP) changes induced by dofetilide (Fig. 1E). Rather, RSD1235 and dofetilide co-treatment tended to increase ERP in an additive manner.

3.3. RSD1235 terminates EADs induced by dofetilide

Early afterdepolarizations (EADs) are membrane potential oscillations that occur at action potential plateau
voltages; they may trigger ventricular arrhythmias [7], and can be induced by drugs that delay AP repolarization, such as clofilium and dofetilide [22]. The ability of RSD1235 to reverse dofetilide-induced AP prolongation in vitro suggests that it may also terminate EADs generated by dofetilide. To test this idea, isolated Purkinje fibers were perfused with 300 nM dofetilide to induce EADs, before adding RSD1235 (Fig. 2). Control APs at 1 and 3 s intervals are shown in Fig. 2A. 300 nM dofetilide increased APD and induced the formation of EADs (Fig. 2B) in all preparations tested (n = 7). When it was no longer possible to pace the preparation at 3 s intervals in the presence of dofetilide, the stimulation rate was reduced to accommodate the increased APD. EADs occurred at mean take-off potentials of −29±1 mV and had trough-to-peak amplitudes of 22±2 mV (n = 61 EADs from 7 experiments). By comparison, APs had mean peak amplitudes of 112±4 mV, with APD<sub>50</sub> occurring at a mean voltage of −28±2 mV (n = 7). The number of EADs was roughly proportional to APD, such that longer APs had more EAD deflections. In the presence of dofetilide, APD continued to increase until 30 μM RSD1235 was added to the perfusate. In all cases (n = 7), when 30 μM RSD1235 was added to the perfusate containing dofetilide, the AP shortened and the number of EADs declined. After a mean duration of 35±8 min in the presence of dofetilide+RSD1235, EADs were terminated in all experiments, and the APD returned to near pre-drug durations (Fig. 2C).

![Fig. 3. RSD1235 blocks multiple components of I<sub>Na</sub> during step depolarization and ramp repolarization voltage protocols. A. Above: The voltage protocol consisted of a 100 ms step depolarization from −100 to +20 mV, followed by a 100 ms ramp from +20 back to −100 mV. Below: The transient component of Nav1.5 currents (I<sub>early</sub>) in control (pre-drug), in the presence of 30 μM RSD1235, and in the presence of 30 μM TTX plus 30 μM RSD1235. B. Averaged Nav1.5 current traces (45 consecutive traces in each case) following leak subtraction under the same conditions as in A. Note that the time base here is much longer than in A, and includes repolarization currents during the voltage ramp. C, D. After digital subtraction (see Methods), the RSD1235- and TTX-sensitive components of I<sub>early</sub> in panel C, and the sustained (I<sub>sus</sub>) and late (I<sub>late</sub>) components in panel D, are revealed. Note that I<sub>early</sub> is off-scale in B and D.](image-url)
3.4. RSD1235 blocks a late sodium current

The effects upon APD₉₀ suggested that RSD1235 was effective at promoting repolarization at critical plateau potentials. Inhibition of inward current could speed repolarization and although RSD1235 does not block L-type Ca²⁺ current, it does inhibit Nav1.5 expressed in myocytes and HEK cells [3]. Other Na channel blockers like lidocaine, flecainide and mexiletine can inhibit a late inward and HEK cells [3]. Other Na channel blockers like lidocaine, flecainide and mexiletine can inhibit a late inward and HEK cells [3].

RSD1235 ramped back to effective at blocking the late current than the early current (Fig. 4A). Lidocaine, a drug that, like RSD1235, terminated dofetilide-induced EADs, also blocked a significantly greater proportion of Iₜₙₐₑₜ (70±2.5%) than Iₑₜₚₑₚₐₓ (41±2.7%) (p<0.01), but in the presence of lidocaine, Iₑₜₚₑₚₐₓ was also more significantly blocked than Iₑₜₚₑₚₐₓ (p<0.01, Fig. 4B).

3.5. Lack of Tdp inducibility and prevention of clofilium-induced Tdp by RSD1235

In the modified Carlsson model of Tdp, rabbits treated with the alpha₁-adrenergic agonist methoxamine and the class III agent clofilium consistently demonstrated QT prolongation, bradycardia, premature ventricular contractions (PVCs) (Fig. 5B), and Tdp in 7 of 9 animals, as exemplified in the bottom ECG strip. In these control animals, Tdp occurred at a median time of 7.0±1.3 min after starting clofilium infusion, at a cumulative dose of 1.4±0.2 mg/kg clofilium. The first PVC occurred at a median time of 2.9 min after starting clofilium infusion and no arrhythmias were observed during the interval when methoxamine was infused alone.

When continuous infusion of RSD1235 was started before methoxamine and clofilium, the time to first PVC increased and Tdp inducibility declined, so that at doses of 1 μmol/kg/min, often no arrhythmias were observed (Figs. 5C and 6A). There was no difference in heart rate during the methoxamine and clofilium infusions between the control group and those animals additionally treated with RSD1235, indicating that the decline in Tdp inducibility was not due to overdrive suppression nor alpha-1 antagonism by the compound. The overall arrhythmia severity and frequency of Tdp was reduced in RSD1235 pre-treated animals to 1 of 9 animals (p<0.05) at 1 μmol/kg/min. Lower doses of RSD1235 (0.1 and 0.3 μmol/kg/min) did not significantly affect the number of animals developing one or more episodes of Tdp. Time to first PVC was delayed from...
Fig. 6. Incidence and duration of clofilium-induced TdP are reduced in RSD1235 pre-treated rabbits. A. Incidence of TdP in control rabbits infused with methoxamine and clofilium (concentrations as in Fig. 5 legend), compared with test animals additionally pre-treated with an RSD1235 infusion begun 5 min before methoxamine. Numerators indicate the number of animals in which TdP occurred and denominators represent the total number of animals tested. \( p < 0.05; \) Fishers Exact test. B. Duration of TdP in control and in RSD1235 pretreated animals. Bar amplitudes indicate the mean duration of TdP episodes per animal in each group. Control and test animals are the same as in A, \( n = 9 \) in each case. Stars denote significant difference compared to control: \( * p < 0.05. \) Error bars are \( \pm \)SEM.

Fig. 5. Experimental timelines and ECG tracings from a control and a rabbit pre-treated with RSD1235. A. Protocol for pretreatment experiments. Bars indicate the timeline of drug infusions during complete experiments. All infusions were terminated after 35 min of recording. Methoxamine was infused at 20 \( \mu \)g/kg/min, clofilium at 300 nmol/kg/min, and RSD1235 at 0.1, 0.3, or 1 \( \mu \)mol/kg/min. B. Rabbit ECG recordings pre-drug, 10 min after starting methoxamine infusion, 1 min after clofilium was added to the infusion regimen, and after induction of torsades de pointes (TdP). Premature ventricular contractions (PVCs) were frequently observed during clofilium infusion. C. ECG recordings pre-drug, 5 min after starting RSD1235 infusion (1 \( \mu \)mol/kg/min), 10 min after methoxamine was added to the infusion, and 15 min after clofilium was added to the infusion.
median values of 2.1 min at 0.1 μmol/kg/min, to 3.9 min at 0.3 μmol/kg/min, and 6.9 min at 1 μmol/kg/min (p < 0.05). Since TdP occurred in runs that often self-terminated, the effect of RSD1235 on the duration of the episodes of TdP during the 15 min clofilium infusion was also examined. In RSD1235-pretreated animals the mean summed duration of TdP episodes per animal was significantly reduced from 71±23 s in controls (n = 9) to 14±14 s (n = 9; p < 0.001) at a dose of 1 μmol/kg/min RSD1235 (Fig. 6B). The effects of 0.1 and 0.3 μmol/kg/min on clofilium-induced TdP duration were 98±24 and 17±7 s, respectively, (n = 9; p > 0.05). RSD1235 and methoxamine, administered for 10 min, induced no arrhythmias, suggesting that RSD1235 has no tendency to induce TdP itself in this model. A potential mechanism for TdP prevention by RSD1235 is the suppression of QTc prolongation. QTc was measured at 5-min intervals for the duration of the experiment as shown in Fig. 7. In control rabbits the QTc interval increased by 119±26% from that measured just before clofilium infusion. In rabbits pretreated with 0.1, 0.3 or 1 μmol/kg/min RSD1235, QTc increased by 92±30%, 46±7% (p < 0.05), and 24±6% (p < 0.01) in the presence of clofilium.

3.6. Acute termination of episodes of TdP by RSD1235

In animals treated with methoxamine and clofilium, which resulted in TdP, subsequent infusions of RSD1235 reduced the overall duration of TdP. 1 or 3 μmol/kg/min RSD1235 was infused over 5 min (corresponding to cumulative doses of 1.2 or 5.8 mg/kg) immediately after the first episode of TdP as shown schematically in Fig. 8A. 5.8 mg/kg RSD1235 reduced the overall duration of TdP from 75±8 s in control (n = 9) to 17±9 s (p < 0.05) and restored sinus rhythm in all animals by the end of the infusion (n = 9, Fig. 8B). 1.2 mg/kg of RSD1235 reduced TdP duration to 35±15 s and restored sinus rhythm in 2 of 9 animals. The decline in TdP duration after RSD1235 infusion began was dependent on infusion duration (Fig. 8C). At both infusion rates of RSD1235, there was no effect on TdP duration in the first minute of infusion. However, in the 2nd to 5th minute, TdP duration significantly declined at the higher dose. At the lower dose TdP decreased only in the 3rd and 5th minute compared to controls at the same time points.

4. Discussion

RSD1235 had minor effects on rabbit Purkinje fiber action potentials (APD50 and APD90) at concentrations around those necessary to terminate AF in humans (peak plasma levels of 5.8 μg/ml, ~15 μM, [4]), and at higher concentrations it reversed the APD prolongation and terminated EADs induced by dofetilide (Figs. 1, 2). RSD1235 blocked late Na+ channel activity during repolarizing ramps and this was correlated with Na+ current active at EAD take-off potentials during AP repolarization (Figs. 3, 4). In vivo, RSD1235 was clearly able to both prevent and terminate TdP induced by an archetypal class III agent, clofilium (Figs. 5–8).

4.1. Mechanism of anti-TdP actions

Pretreatment with RSD1235 prevented the onset of, and shortened the duration of TdP in rabbits exposed to methoxamine and clofilium (Figs. 5, 6). This was associated with a significant normalization of QTc (Fig. 7). In agreement with this, RSD1235 lacked substantive actions on Purkinje fiber APD50, APD90, or ERP, but was able to attenuate the APD-prolonging actions of dofetilide (Fig. 1B). The lack of major effect on rabbit Purkinje fiber APD at RSD1235 concentrations between 0.3–30 μM (Fig. 1) despite the ability of the drug to block repolarizing outward currents [3] suggested that an inward current was also blocked by RSD1235. We have attributed these effects to late INa blockade [25]. In Purkinje fiber preparations in which IKr was inhibited by dofetilide, RSD1235 was particularly effective at promoting repolarization at APD50 voltages and terminating EADs with take-off potentials at or near APD50 voltages of ~30 mV (Figs. 1, 2).

INa, as well as being responsible for the rapid AP upstroke, also plays a critical role during AP repolarization [26,27]. Studies have demonstrated that Nav1.5 current increases during AP repolarization and that a slight enhancement of this current component, Ilate, may be

![Fig. 7. QTc interval in control and RSD1235 pre-treated animals. Horizontal bars indicate the infusion of drug. QTc interval in animals receiving no pre-treatment (control) and in animals pre-treated with 0.1, 0.3 or 1 μmol/kg/min RSD1235. After starting clofilium infusion, QTc measurements were confounded by an escalating frequency of arrhythmias. As a result, the animals were dropped from the sample when measurements could not be made at that interval. Sample size at each time point is indicated by the numbers above the graph. Stars denote significant difference compared to values just before clofilium infusion: *p < 0.05; **p < 0.01. Data points are mean±SEM.](image-url)
sufficient to induce EADs [28]. \( I_{\text{late}} \) is not a window current, since the voltages at which it peaks (−24±3.4 mV) are outside the range of the inactivation/activation curve overlap. Rather, this current has been attributed to recovery of Nav1.5 channels from inactivation occurring during a negative plateau, along with an increased driving force for Na⁺ entry as repolarization develops. Mutation of key amino acid residues that sped up recovery from inactivation (while not affecting steady-state inactivation), selectively increased \( I_{\text{late}} \) [26].

EADs are induced by intracellular calcium oscillations that arise from an imbalance between sarcolemmal Ca²⁺ entry and release from sarcoplasmic reticulum stores [7,8,22]. Treatments that facilitate sarcolemmal Ca²⁺ entry increase the potential for EAD induction. Simulations using a Luo–Rudy model indicated that when \( I_{\text{late}} \) is selectively increased two-fold, enough inward current is present during repolarization to trigger EADs [26]. In our experiments, block of \( I_{\text{Kr}} \) in Purkinje fibers by dofetilide resulted in an unopposed \( I_{\text{late}} \) which depolarized the membrane sufficiently to tip the balance in favor of EAD formation (Fig. 2B). In support of this, EADs occurred at similar potentials to those at which \( I_{\text{late}} \) peaked, i.e., −29 and −24 mV, respectively (compare Figs. 2B and 3). These results suggest that \( I_{\text{late}} \), despite being less than 1% of \( I_{\text{early}} \), plays a facilitatory role in EAD formation. RSD1235 was effective at terminating EADs (Fig. 2C) and both RSD1235 and lidocaine induced proportionally more block of \( I_{\text{late}} \) than \( I_{\text{early}} \) (Fig. 4). Thus, block of \( I_{\text{late}} \) is a reasonable explanation for RSD1235’s action against class III-induced instability of repolarization, although the use of a single, supratherapeutic concentration of RSD1235 in these experiments represents a limitation with regard to the clinical implications of our findings. As well, we cannot exclude the contribution of other Na⁺...
channel blocking properties of RSD1235 to the prevention or termination of TdP. Rate-dependent enhancement of $I_{Na}$ inhibition by RSD1235 may prevent rapid depolarizations observed during TdP, and enhanced block of $I_{Na}$ at depolarized voltages may inhibit EAD deflections from the AP plateau [23].

4.2. Clinical implications

Based on the experiments performed in this study, RSD1235 is predicted to have a low potential for inducing ventricular arrhythmias. At therapeutic concentrations, the K$^+$ channel blocking actions outweigh the Na$^+$ channel actions of RSD1235 [3], so in conversion of atrial fibrillation, it is unlikely to suffer from the proarrhythmic effect of conduction slowing in normal or ischemic tissue. At high concentrations (30 $\mu$M, Fig. 1E) RSD1235 did significantly prolong the APD90 and ERP, and the latter effects were additive to those of dofetilide. However, this action is not expected to be significant at clinically used concentrations [1,4]. As well, in the present studies, RSD1235 only minimally prolonged Purkinje fiber APD at concentrations that terminate AF [4], and had a suppressive action on EADs. Clinically, the use of $I_{Kr}$ blockers such as dofetilide and sotalol in the treatment of atrial flutter and fibrillation [5,29,30] is complicated by the likelihood of proarrhythmic episodes, typically TdP [31]. The results indicated that RSD1235’s electrical interactions with class III antiarrhythmics were benign, and that RSD1235 could terminate episodes of TdP induced by class III agents. RSD1235 infused before clofilium prevented the induction of arrhythmias (Figs. 5, 6) and terminated TdP when started after the first episode of TdP (Fig. 8). RSD1235 attenuated class III-induced APD prolongation and EAD genesis (Figs. 1, 2), and interestingly, the sequence of drug addition played an important role in determining the extent to which RSD1235 attenuated the effects of dofetilide in vitro. When dofetilide was added to the bathing solution first, RSD1235’s ability to reverse the APD prolongation was not as great as RSD1235’s ability to prevent AP prolongation when it was perfused before dofetilide (Fig. 1C, D). This effect was mirrored in the in vivo experiments in which greater infusion rates of RSD1235 were required to completely terminate TdP than were required to prevent it (Figs. 6, 8). While the effect of RSD1235 on dofetilide-induced APD prolongation was not additive, the effect on ERP was additive. This may be reflective of RSD1235’s peak $I_{Na}$-blocking action. Co-treatment with RSD1235 and dofetilide enhanced refractoriness above that of either drug alone (Fig. 1E). These results suggest that use of RSD1235 is not likely to complicate administration of class III antiarrhythmic agents. As well, this class of drug may in the future be useful in treating human polymorphisms that result in long QT (LQT) syndromes, such as LQT2 (hERG polymorphism) and LQT3 (Na$^+$ channel polymorphism), in which patients experience greatly increased susceptibility to TdP [32,33].

4.3. Conclusions

In this study, in vitro data support the clinical finding that RSD1235 has only minimal effects on ventricular repolarization at therapeutic concentrations. RSD1235 was effective in reversing AP prolongation in Purkinje fibers and in terminating the EADs that occurred during exposure to dofetilide. RSD1235 was also safely co-administered with class III antiarrhythmics in the in vivo rabbit model, while proving an effective pre-treatment and/or remedy for TdP induced by drugs that prolong ventricular repolarization. In voltage ramp experiments, a component of late $I_{Na}$ active during the AP plateau was inhibited by RSD1235, and this may explain at least in part, at the single (30 $\mu$M) concentration used in the present experiments, its ability to suppress both EADs and TdP.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.cardiores.2006.01.026.

References