Antiarrhythmic properties of a rapid delayed-rectifier current activator in rabbit models of acquired long QT syndrome

Thomas G. Diness1,2†, Yung-Hsin Yeh1,3†, Xiao Yan Qi1, Denis Chartier1, Yukiomi Tsuji4, Rie S. Hansen2, Soren-Peter Olesen2, Morten Grunnet2, and Stanley Nattel1*

1Department of Medicine and Research Center, Montreal Heart Institute and Université de Montréal, 5000 Belanger Street East, Montreal, Quebec, Canada H1T 1C8; 2NeuroSearch A/S and Danish National Research Foundation Centre for Cardiac Arrhythmia, University of Copenhagen, Copenhagen, Denmark; 3First Cardiovascular Division, Chang Gung Memorial Hospital, Chang Gung University, Taoyuan, Taiwan, Republic of China; 4Department of Cardiovascular Research, Institute of Environmental Medicine (RIEM), Nagoya University, Nagoya, Japan

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Aims Impaired repolarization in cardiac myocytes can lead to long QT syndrome (LQTS), with delayed repolarization and increased susceptibility to Torsades de Pointes (TdP) arrhythmias. Current pharmacological treatment of LQTS is often inadequate. This study sought to evaluate the antiarrhythmic effect of a novel compound (NS1643) that activates the rapid delayed-rectifier K\textsuperscript{+} current, I\textsubscript{Kr}, in two rabbit models of acquired LQTS.

Methods and results We used two clinically relevant in vivo rabbit models of TdP in which we infused NS1643 or vehicle: (i) three-week atrioventricular block with ventricular bradypacing; (ii) dofetilide-induced I\textsubscript{Kr} inhibition in methoxamine-sensitized rabbits. In addition, we studied effects on ionic currents in cardiomyocytes with I\textsubscript{Kr} suppressed by bradycardia remodelling or dofetilide exposure. Bradypaced rabbits developed QT interval prolongation, spontaneous ventricular ectopy, and TdP. Infusion of NS1643 completely suppressed arrhythmic activity and shortened the QT interval; vehicle had no effect. NS1643 also suppressed ventricular tachyarrhythmias caused by infusion of dofetilide to methoxamine-sensitized rabbits, and reversed dofetilide-induced QT prolongation. NS1643 increased I\textsubscript{Kr} in cardiomyocytes isolated from normal and bradycardia-remodelled rabbits by approximately 75% and 50%, respectively (P < 0.001 for each). Similarly, NS1643 restored I\textsubscript{Kr} suppressed by 5 nmol/L dofetilide (tail current 0.28 ± 0.03 pA/pF pre-dofetilide, 0.20 ± 0.01 pA/pF in the presence of dofetilide, 0.27 ± 0.02 pA/pF after adding NS1643 to dofetilide-containing solution, P < 0.01).

Conclusion Pharmacological activation of I\textsubscript{Kr} reverses acquired LQTS and TdP caused by bradycardia remodelling and I\textsubscript{Kr}-blocking drugs. I\textsubscript{Kr}-activating drug therapy could be a potentially interesting treatment approach for LQTS.

1. Introduction

Long QT syndrome (LQTS) is caused by impaired ventricular repolarization associated with action potential duration (APD) prolongation, manifesting as an abnormally long QT interval and ventricular arrhythmias. LQTS can be inherited as an autosomal dominant (Romano-Ward syndrome)\textsuperscript{1,2} or recessive (Jervell and Lange-Nielsen syndrome)\textsuperscript{3} disorder due to loss-of-function mutations in cardiac K\textsuperscript{+}-channel genes such as HERG, KCNQ1, KCNE1, or KCNE2 or gain-of-function mutations in the cardiac Na\textsuperscript{+} or Ca\textsuperscript{2+}-channel genes, SCN5A or CACNA1C.\textsuperscript{4} Often, however, LQTS is acquired upon exposure to drugs that block cardiac K\textsuperscript{+}-channels and/or cardiac disease.\textsuperscript{5} Delayed repolarization favours the genesis of early after-depolarizations (EADs) and associated polymorphic Torsades de Pointes (TdP) tachyarrhythmias. Repolarization-impairing drugs and mutations amplify the electrical heterogeneities intrinsic to the ventricular myocardium, increasing the dispersion of repolarization, and creating a substrate for reentry.\textsuperscript{6}

A variety of clinical approaches have been used to treat LQTS. Therapy of congenital LQTS presently reposes on two modalities: β-adrenoceptor blockers, which are most...
effective in Type 1-LQTS, and implantable defibrillators. Acquired LQTS is treated with intravenous (i.v.) magnesium sulfate, overdrive pacing, and occasionally isoproterenol. Certainly, there is room for improvement in treating LQTS: β-blockers and magnesium sulfate are often ineffective, implanted defibrillators can cause a variety of complications and frequent discharges can be a problem. One potentially interesting approach to LQTS therapy would be the use of a drug that directly targets the underlying physiological dysfunction, a deficiency in net repolarizing current. Several K⁺-channel agonist drugs have been developed recently, among which drugs like NS1643 that increase rapid delayed-rectifier current (I₆) conductance in ERG-expressing cells are of potential interest in LQTS.

Rabbits with complete atrioventricular block (AVB) develop QT prolongation and frequent spontaneous TdP episodes, apparently because of downregulation of ERG/I₆. Similarly, I₆ blockers cause QT prolongation and TdP in rabbits, particularly in the presence of the α-adrenoceptor agonist methoxamine, producing a model that shares many features with clinical drug-induced LQTS. The present study was designed to investigate the ability of acute NS1643 administration to reverse the electrophysiological and proarrhythmic manifestations of these two models of I₆-related acquired LQTS in vivo, and to relate any actions to changes in the corresponding I₆ recordings in vitro.

2. Methods
Animal handling followed the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996); all procedures were approved by the Animal-Experimentation Ethics Committee of the Montreal Heart Institute and University of Copenhagen. Female New Zealand white rabbits were used for all studies.

2.1 Whole-cell patch-clamp
Rabbit ventricular myocytes were obtained and isolated as described previously. After cervical dislocation, hearts were quickly excised and perfused in Langendorff mode with oxygenated Ca²⁺-free Tyrode’s solution at 38 °C for 5 min, followed by perfusion with Ca²⁺-free Tyrode’s solution containing 0.8% collagenase type 2 (Worthington) for 20–30 min. Tyrode’s solution contained (mmol/L): NaCl, 136; KCl, 5.4; MgCl₂, 1; CaCl₂, 1; NaH₂PO₄, 0.33; HEPES 5; dextrose 10 (pH 7.35, NaOH). Isolated cardiomyocytes were then quickly excised and perfused in Langendorff mode with oxygenated Ca²⁺-free extracellular solutions containing (mmol/L): KCl, 20; KH₂PO₄, 10; dextrose, 10; mannitol, 40; l-glutamic acid, 70; β-OB-butryic acid, 10; taurine, 20; EGTA, 10; 0.1% bovine serum albumin (pH 7.3, KOH). Currents were recorded with whole-cell patch-clamp at 36 ± 0.5 °C with Na⁺- and K⁺-free extracellular solutions containing (mmol/L): NaCl, 149; NaH₂PO₄, 5; HEPES 5; CaCl₂, 0.9 to eliminate I₆ and inward-rectifier K⁺-current (I₇). L-type Ca²⁺-current was blocked with 10 μmol/L of nifedipine. The pipette solution contained (mmol/L): K⁺-aspartate, 110; KCl, 20; MgCl₂, 1; Mg²⁺-ATP, 1; Li⁺-GTP, 0.1; HEPES, 10; Na⁺-phosphocreatine, 5; EGTA, 10 (pH 7.3 with KOH). Borosilicate-glass electrodes (tip resistances of 1.5–3.0 MΩ) were used to record whole-cell currents. Cell capacitance and series resistance were compensated by approximately 55–70%. I₆ was recorded in the presence of the highly selective I₆ blocker HMR-1566 (0.5 μmol/L, Sanofi-Aventis, Frankfurt, Germany). Unless otherwise specified, chemicals were obtained from Sigma Chemicals, St Louis, MO.

2.2 Bradypaced atrioventricular block-rabbit model
Rabbits (1.8–2.5 kg) were anesthetized with intramuscular (i.m.) ketamine (35 mg/kg) and xylazine (10 mg/kg). They were then artificially ventilated with O₂-enriched air containing 2% isoflurane. AVB was created and a programmable unipolar right ventricular pacemaker (Vitatron, Arnhem, The Netherlands) was implanted as previously described. Rabbits received penicillin-G benzathine 75 000 IU + penicillin-G procaine 75 000 IU i.m. pre-operatively and two days post-operatively, as well as 78 μg of subcutaneous buprenorphine immediately post-operatively and 60 μg the next day. AVB-rabbits were paced initially at 130 b.p.m. for three days. The pacing rate was decreased to 90 b.p.m. on day 3 and 60–90 b.p.m. on day 7. If a ventricular escape rhythm faster than 90 b.p.m. was observed, rabbits were demand-paced at 40 b.p.m.

Lead-II echocardiograms (ECGs) were recorded under ketamine (25 mg/kg)/xylazine (5 mg/kg) anaesthesia on days 3, 7, 14, and 21 post-operatively. When recurrent TdP and/or frequent ventricular ectopy were observed, rabbits were re-anasthetized with i.m. ketamine/xylazine (25/5 mg/kg initially, followed by 17/3 mg/kg every 30 min). After 30 min of stabilization, baseline arrhythmias were recorded for 9 min. Thereafter, NS1643 (1,3-Bis-(2-hydroxy-5-trifluoromethyl-phenyl)-urea) synthesized in-house at NeuroSearch A/S, Ballerup, Denmark (1.5 mg/kg/min) or vehicle (50% polyethylene glycol 400/50% isotonic glucose at the equivalent infusion rate, 2 mL/kg/h) was infused for 45 min (Supplementary material online, Figure S1A). QT intervals were measured every third minute, as an average of four successive QT intervals, and corrected for heart rate with the mean of the four corresponding RR intervals. The rate-corrected QT (QTc) was calculated according to Carlson’s formula: QTc = QT – 0.175(RR – 300).

To study effects on dofetilide-induced arrhythmias, a methoxamine-sensitized rabbit model was adapted from Carlson et al. Rabbits (2.8–3.2 kg) were anasthetized with i.m. ketamine/xylazine (35/7 mg/kg followed by 17/3 mg/kg every 30 min). Lead-II ECGs were recorded. Methoxamine in isotonic saline solution was infused at 17 μg/kg/min. Simultaneously, vehicle or NS1643 (1.2 mg/kg/min) were infused. Beginning 15 min later, dofetilide in acidified saline solution (pH 4) was co-infused for 35 min at 0.03 mg/kg/min (Supplementary material online, Figure S1C).

2.4 Data analysis
Clampfit 6.0 (Axon Instruments, Foster City, CA) and GraphPad Prism 4 (GraphPad, San Diego, CA) were used for data analysis. All data are expressed as mean ± SEM. A P < 0.05 was considered statistically significant. For I-V curves, two-way repeated measures analysis of variance (ANOVA) was performed using mixed model methodology with NS1643 and membrane potential as main effects. In the case of a significant interaction between the two main effects, Bonferroni-corrected t-tests were used to compare individual mean values. The mixed procedure and multiple comparisons were used to evaluate differences in current amplitudes at different membrane potentials in the absence and presence of NS1643. Current amplitudes in the absence of NS1643 and NS1643/dofetilide were compared with baseline of one-way ANOVA followed by T ukey’s post-test. AVB and doxepin were evaluated the same way. Non-linear curve fitting and

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linear regression were performed by least-squares methods. In methoxamine-sensitized rabbits, two-way ANOVA was used to evaluate the effect of dofetilide infusion and of NS1643 on ectopic beat and ventricular tachycardia (VT) prevalence.

3. Results

3.1 Effects on \( I_{Kr} \) under conditions causing acquired long QT syndrome

Reductions in \( I_{Kr} \) due to transcriptional downregulation appear to be an important underlying cause of repolarization and rhythm abnormalities in the AVB-rabbit model.\(^{10}\) To determine whether NS1643 can restore \( I_{Kr} \) current density in this model, we studied the effect of the drug on ventricular cardiomyocytes. Figure 1A shows \( I_{Kr} \) recordings before and after 10 \( \mu \)mol/L NS1643. NS1643 increased currents activated by the depolarizing pulse, as well as tail currents recorded upon repolarization. In control rabbits, NS1643 significantly increased the density of \( I_{Kr} \) tails recorded at \(-50 \text{ mV} \) for all test-pulse voltages between 0 and \(+50 \text{ mV} \) (Figure 1B). For example, for a step to \(+50 \text{ mV} \), tail currents increased by approximately 75%, from 0.28 ± 0.08 to 0.49 ± 0.12 pA/pF, \( P < 0.001 \). NS1643 did not significantly change voltage-dependence of \( I_{Kr} \) activation (Figure 1C): \( V_{1/2} \) was \(-4.5 \pm 2.3 \text{ mV} \) and \(-7.7 \pm 2.6 \text{ mV} \) before and after NS1643, respectively (\( P = \text{NS} \)). Similarly, deactivation time-constants were unaltered: slow and fast time-constants at \(-50 \text{ mV} \) averaged 917 ± 102 and 122 ± 13 ms, respectively, in the absence and 872 ± 159 and 107 ± 5 ms in the presence of NS1643 (\( n = 5 \) cells/group, \( P = \text{NS} \) for both). Qualitatively similar results were obtained in cardiomyocytes from AVB-rabbits (Figure 1D and E). For example, following steps from \(+50 \) to \(-50 \text{ mV} \), the tail current amplitude increased by approximately 50%, from 0.20 ± 0.05 to 0.30 ± 0.05 pA/pF, \( P < 0.001 \) (Figure 1D), returning values to the same range as the average control current in normal-rabbit cardiomyocytes. As in normal-rabbit cells, activation voltage-dependence and deactivation kinetics were not influenced by NS1643. \( V_{1/2} \) averaged \(-5.0 \pm 2.2 \text{ mV} \) and \(-3.2 \pm 2.3 \text{ mV} \) before and after NS1643, respectively (\( P = \text{NS} \) for both). Deactivation time-constants averaged: \( \tau_{\text{slow}} \) 899 ± 116 vs. 789 ± 103 (\( n = 6 \), \( P = \text{NS} \)); \( \tau_{\text{fast}} \) 148 ± 20 vs. 144 ± 27 ms (\( n = 6 \)/group, \( P = \text{NS} \)) in the absence and presence of NS1643, respectively.

The studies described above indicate that NS1643 can restore \( I_{Kr} \) that has been reduced by downregulation of \( I_{Kr} \) expression. We then addressed the issue of whether NS1643 can restore \( I_{Kr} \) that has been suppressed by pharmacological blockade. Isolated ventricular myocytes were exposed to 5-nmol/L dofetilide and, subsequently, to 10 \( \mu \)mol/L NS1643 in the continued presence of 5 nmol/L dofetilide. Figure 2A shows the time-dependent response in one cardiomyocyte, with corresponding original recordings shown in Figure 2B. Dofetilide reduced \( I_{Kr} \), which was

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Figure 1 Effects of NS1643 on \( I_{Kr} \) in isolated ventricular cardiomyocytes from normal and atrioventricular-blocked (AVB) rabbits. (A) Representative \( I_{Kr} \) recordings at baseline and after superfusion with 10 \( \mu \)mol/L NS1643. (B) \( I-V \) curves for \( I_{Kr} \) tail currents at baseline and after 10 \( \mu \)mol/L NS1643 in control rabbits. NS1643 significantly increased \( I_{Kr} \) at all voltages \( \geq 0 \text{ mV} \). **\( P < 0.05 \), ***\( P < 0.01 \), ****\( P < 0.001 \) vs. baseline at same voltage. (C) Voltage-dependent activation in control rabbits based on normalized tail currents. Data points were fitted by Boltzmann functions. All data in (B) and (C) are from \( n = 5 \) cells isolated from three different control rabbits. (D) \( I_{Kr} \) tail currents at baseline and after 10 \( \mu \)mol/L NS1643 in AVB-rabbit cardiomyocytes. **\( P < 0.01 \), ****\( P < 0.001 \) vs. baseline at same voltage. (E) Voltage-dependent activation in AVB-rabbit cells. Boltzmann function fits are shown. All data in (D) and (E) are from \( n = 5 \) cells isolated from three different AVB-rabbits.
Effects of NS1643 on dofetilide-suppressed \( I_{Kr} \) in ventricular cardiomyocytes from normal rabbits. (A) \( I_{Kr} \) tail current densities at baseline, after superfusion of 5 nmol/L dofetilide, and subsequent co-superfusion of 5 nmol/L dofetilide and 10 \( \mu \)mol/L NS1643 in one representative experiment. (B) Examples of original tail currents recorded at the end of baseline measurements (a), the end of dofetilide-only infusion (b), and at the end of dofetilide plus NS1643 superfusion (c) in the experiment illustrated in (A). (C) Mean ± SEM \( I_{Kr} \) tail current densities at −50 mV following a depolarizing pulse to +50 mV (** \( P < 0.01 \)).

then restored by NS1643. Overall, 15 min exposure to dofetilide significantly reduced \( I_{Kr} \) by approximately 30%, from 0.28 ± 0.03 to 0.20 ± 0.01 pA/pF (Figure 2C). The subsequent co-application of NS1643 increased dofetilide-suppressed \( I_{Kr} \) by approximately 35%, from 0.20 ± 0.01 to 0.27 ± 0.02 pA/pF.

NS1643 can inhibit currents carried by KCNQ1/KCNE1 co-expressed subunits in Xenopus oocytes. Since one of our objectives was to relate in vitro findings to in vivo effects, we determined whether NS1643 alters \( I_{Kr} \) in rabbit cardiomyocytes. \( I_{Kr} \) was recorded with the voltage protocol illustrated in Figure 3A. The same solutions were used as for \( I_{Kr} \) recording, but HMR-1566 was replaced by 1 \( \mu \)mol/L dofetilide (Sequioa Pharmaceuticals, Gaithersburg, MD) in the superfusate. Robust \( I_{Kr} \) was recorded both before and after NS1643 superfusion (Figure 3A), and neither \( I_{Kr} \) step (Figure 3B) nor tail (Figure 3C) current densities were affected by NS1643.

3.2 Effects on arrhythmic activity in bradypaced rabbits

The left panel in Figure 4A shows an example of ventricular arrhythmias recorded from a bradypaced rabbit. The right panel depicts superimposed lead-II ECG recordings from an arrhythmic bradypaced rabbit before and after 35 min of infusion of NS1643. Grey and black lines indicate, respectively, ECGs recorded at baseline and during NS1643 infusion. QT-interval abbreviation by NS1643 is evident at the upper left. Prior to drug infusion, ventricular ectopic activity and short runs of TdP arose from the T-wave; no such events were observed in this rabbit after NS1643-administration.

Arrhythmic rabbits were randomly assigned to either vehicle or NS1643 groups (\( n = 6 \) each). Baseline ectopic-complex frequency (Figure 4B) did not differ between groups. Rabbits receiving vehicle showed no statistically significant time-dependent change. In contrast, the NS1643-infused group displayed a significant decrease in ectopic-complex prevalence beginning 15 min after infusion onset. The antiarrhythmic effect persisted throughout the infusion, with no ectopic complexes observed in the NS1643 treated group from the 24th minute (Figure 4B).

Because of frequent ectopic activity and TdP, it was possible to measure the QT interval throughout the experimental period in only four of the six arrhythmic rabbits in each of the groups. Four successive QT intervals were measured in each of the three-minute intervals during which ectopic beats were counted. Means during the 9 min before infusion-onset were used as baselines. There were no statistically significant differences in baseline QT-values, with a baseline QT interval of 371 ± 11 ms (Figure 4C), slightly longer than the value reported by Tsuji et al. of approximately 350 ms. The vehicle-infused group showed an initial small QT-interval increase, with values returning to baseline thereafter. In the NS1643 group, statistically significant QT-interval decreases began 21 min after infusion-onset and progressed throughout the rest of the experiment.

We then sought to determine whether the antiarrhythmic effect of NS1643 was quantitatively related to QT-interval shortening, comparing arrhythmia and QT-interval changes in the four rabbits/group in which they could both be followed for most of the infusion period (Supplementary material online, Figure S2A and B), thus showing that the overall time course of arrhythmia suppression paralleled that of QT-interval changes in these rabbits. NS1643-induced statistically significant effects on ectopic-complex frequency (Supplementary material online, Figure S2A) and QT intervals (Supplementary material online, Figure S2B) began at the same time, the 24th minute of drug infusion. Quantitatively, changes in ectopic-complex frequency appear to be larger over the first 20 min than changes in QT interval. The relationship between QT interval and ectopic-complex frequency changes caused by the drug was pursued (Supplementary material online, Figures S2C and D), in an analysis of ectopic-beat frequency as a function of QT intervals (SEMs are omitted for clarity). Lines connecting points indicate the time-course of infusion. To address the correlation between arrhythmic activity and QT intervals, a log transformation was performed on the number of ectopic beats (\( > 1 \)) and regression obtained (Supplementary material online, Figure S2D). For the vehicle-group, \( r^2 = 0.13 \) and the slope did not significantly deviate from 0 (95% confidence interval: −0.003 to 0.014). In the NS1643-group, \( r^2 = 0.90 \) and the slope (0.023 ± 0.003) significantly deviated from 0 (95% confidence interval 0.015–0.030), indicating a positive relationship between QT...
interval and ectopic-complex frequency and suggesting that the antiarrhythmic effect was related to QT-shortening.

3.3 Effects on dofetilide-induced QT-prolongation

We next addressed the effects of NS1643 on dofetilide-induced repolarization delays. Dofetilide caused a rapid increase in QT, RR, and QTc intervals (Figure 5A–C). NS1643 infusion progressively decreased QT and QTc intervals, with statistically significant reductions beginning at 30 and 36 min, respectively, after infusion onset (Figure 5A and C). QT and QTc intervals were not significantly altered by vehicle infusion. NS1634 also reversed dofetilide-induced RR-interval changes, but RR-intervals began to increase again after about 70 min (Figure 5B).

3.4 Effects on dofetilide-induced Torsades de Pointes

In preliminary studies, the high mortality rate and variable response to dofetilide of methoxamine-sensitized rabbits proved to be a major obstacle to testing the efficacy of NS1643—many rabbits died before they could receive the compound. We therefore studied the ability of NS1643 to prevent dofetilide-induced TdP. Infusion of NS1643 or vehicle was begun at the same time as methoxamine, with dofetilide infusion started 15 min later. Figure 6 presents the results on a single rabbit (top panels) and group-mean (bottom panels) basis. Dofetilide induced TdP and premature ventricular complexes (PVCs) in all six (100%) of the vehicle-infused rabbits. One of these (Rabbit 4V, Figure 6A) died when TdP degenerated into ventricular fibrillation. Of the rabbits infused with NS1643 prior to and during dofetilide infusion, three of the six were completely devoid of arrhythmias (Rabbits 1–3N, Figure 6B), one had limited ectopy during the first 10 min of the dofetilide-infusion period (Rabbit 6N), one had two episodes of VT during the first 10 min (Rabbit 4N), and one had ectopic activity and VT at a level comparable with the controls (Rabbit 5N). Figure 6C and D shows the mean prevalence of PVCs and VT, respectively. NS1643 significantly reduced the prevalence of PVCs and VT ($P < 0.001$ for each, $n = 6$).

4. Discussion

In this study, we investigated the antiarrhythmic effect of pharmacological activation of $I_{Kr}$ in vivo. Two rabbit models representing clinically distinct forms of acquired LQTS, bradycardia-induced remodelling and selective pharmacological block of $I_{Kr}$, were studied. In bradypaced rabbits, NS1643 suppressed arrhythmic activity and normalized the QT interval. In rabbits at risk of arrhythmias caused by dofetilide-induced $I_{Kr}$ block, NS1643 prevented the emergence of arrhythmic activity, and in rabbits with important dofetilide-induced QT-prolongation, NS1643 substantially decreased the QT interval. Consistent with its in vivo
effects, NS1643 increased $I_{Kr}$ in cardiomyocytes from bradycardia-remodelled rabbits and in cells exposed to dofetilide.

4.1 Relationship to previous studies of $I_{Kr}$ activators

To date, descriptions of five ERG-channel activators have been published.\textsuperscript{9,13–16} A variety of mechanisms are responsible for increasing ERG current: RPR260243 and mallotoxin slow ERG-current deactivation,\textsuperscript{13,16} whereas NS1643 and NS3623 accelerate recovery from inactivation and positively shift the voltage-dependence of inactivation.\textsuperscript{9,15,17} NS3623 also slows inactivation. Four of the ERG current activators have been shown to shorten APD in guinea-pig ventricular cardiomyocytes.\textsuperscript{9,13–15} ECG waveforms recorded from Langendorff-perfused guinea-pig hearts and arterially perfused rabbit ventricular wedges show QT-interval shortening, both in the absence and presence of dofetilide.\textsuperscript{13,14}

Our results add to the literature by demonstrating the ability of an $I_{Kr}$ activator to suppress QT-prolongation and ventricular arrhythmias in vivo in two clinically relevant arrhythmia models. Furthermore, we demonstrate restoration of $I_{Kr}$ in corresponding cardiomyocyte models, confirming the underlying ionic mechanism of action.

Activators of a variety of other cardiac K\textsuperscript{+}-channels have previously been studied, including K\textsubscript{ATP} and KCNQ1 activators.\textsuperscript{18,19} Both types of K\textsuperscript{+}-channel openers decrease APD in cardiac myocytes.\textsuperscript{19,20} Cardiomyocytes isolated from rabbits with hypertensive left ventricular hypertrophy develop APD prolongation and EADs upon exposure to dofetilide: superfusion with a benzodiazepine I\textsubscript{Ks} activator reverses APD prolongation and suppresses EADs.\textsuperscript{21} K\textsubscript{ATP} channel activators suppress EADs induced in cardiac Purkinje fibres by caesium, quinidine, and sematilide, and prevent caesium-induced ventricular-tachyarrhythmia induction in rabbits.\textsuperscript{22} Nicorandil, a K\textsubscript{ATP} channel opener and antianginal
agonist in acquired long QT syndrome

Dofetilide-induced RR interval increases. (A) Dofetilide increased the RR interval. NS1643 reversed the group throughout the experiment. (B) Corresponding RR interval measurements. Dofetilide increased the RR interval. NS1643 reversed dofetilide-induced RR interval increases. (C) QTc intervals obtained with Carlson’s formula. *P < 0.05, **P < 0.01 vs. reference values.

4.2 Novel findings and potential significance

This is to our knowledge the first study of an I\textsubscript{Kr}-activating drug in vivo, as well as the first examination of effects of such drugs on native I\textsubscript{Kr} currents in either normal or LQTS model cardiomyocytes. LQTS is a significant cause of sudden cardiac death. In addition to congenital LQTS, which can be caused by mutations of at least 10 different genes\textsuperscript{24} and the well-recognized acquired form caused by I\textsubscript{Kr}-blocking drugs,\textsuperscript{25} similar pathophysiological mechanisms may contribute to sudden cardiac death in other contexts, including congestive heart failure,\textsuperscript{26} diabetic cardiomyopathy,\textsuperscript{27} and hypertrophic heart disease.\textsuperscript{28} The only widely used drug therapy for congenital LQTS is β-blockers, which can reduce the overall risk of syncpe in LQTS patients.\textsuperscript{29,30} β-Blockers are most effective for LQT1 patients, with a significantly higher failure rate in LQT2 and LQT3 syndromes.\textsuperscript{30}

In LQT3 patients, Na\textsuperscript{+}-channel blockers like mexiletine may be useful by selectively blocking plateau Na\textsuperscript{+}-current through abnormally inactivating Na\textsuperscript{+}-channels.\textsuperscript{31} Defibrillators are often used for LQTS patients and have a low failure rate, but have a wide range of potential complications, and in some patients frequent recurrent arrhythmic events requiring shocks are problematic. Emergency therapy of drug-induced TdP is limited, with i.v. magnesium sulfate considered the treatment of choice but with variable efficacy and risk of neuromuscular depression with excessive doses. Our demonstration of the efficacy of an I\textsubscript{Kr}-enhancing drug in two clinically relevant experimental paradigms suggest that this type of approach could be an interesting potential addition to the clinical armamentarium for LQTS, particularly for patients with an inadequate response to conventional therapy, and one that warrants further investigation.

The LQTS subtype-specificity of I\textsubscript{Kr}-enhancing compounds remains to be determined. In the present study, we have used NS1643 in models of LQTS associated with I\textsubscript{Kr} dysfunction. It is most likely that NS1643 acts by altering the biophysical properties of the remaining functional I\textsubscript{Kr}-channels to acutely enhance their function. In unpublished studies in heterologous expression systems, we have found that NS1643 fails to rescue trafficking-deficient HERG mutants and that complete I\textsubscript{HERG} block by very high dofetilide concentrations is not reversed by NS1643. These results suggest that NS1643 efficacy in LQT2 or acquired LQTS related to I\textsubscript{Kr}-deficiency may be limited to cases with residual I\textsubscript{Kr}-function. On the other hand, NS1643 might also be effective in LQT1 and LQT3 patients, as an agent to increase repolarization reserve through functional I\textsubscript{Kr} channels.

4.3 Potential limitations

I\textsubscript{Kr}-encoding mRNA is present in the sinus node of rabbits\textsuperscript{32} and blocking I\textsubscript{Kr} suppresses the firing rate.\textsuperscript{33} We did observe significant heart-rate acceleration with NS1643, which could contribute to acceleration of repolarization but which could also lead to adverse effects. In addition, excess I\textsubscript{Kr} agonism could be potentially arrhythmogenic if repolarization is excessively accelerated. Vigilance will be needed in clinical trials to detect such complications, if they arise.

The ideal I\textsubscript{Kr} agonist for use in LQTS syndrome would be devoid of other actions. Potential effects of NS1643 have been tested on heterologously expressed KCNQ1, KCNQ1/KCNNE1, Kv1.5, and Kv4.3 channels, as well as L-type Ca\textsuperscript{2+} and Na\textsuperscript{+} currents from guinea-pig ventricular myocytes.\textsuperscript{9} NS1643 has a minor blocking effect on KCNQ1 and KCNQ1/KCNNE1 currents in Xenopus oocytes. In addition, NS1643 also slightly reduces Kv4.3 current. However, these effects...
are small and occur at higher concentrations than those that enhance ERG current. It is unlikely that these collateral actions contributed to the antiarrhythmic effect of NS1643 that we observed, since if anything they would be expected to delay repolarization. Nevertheless, if further investigation supports the potential value of $I_{Kr}$ agonism for the treatment of arrhythmias related to delayed repolarization, chemical development to create more potent and selective $I_{Kr}$ agonists might be of potential interest.

NS1643 had significant (albeit short-lasting) effects on heart rate (Figure 5B), transiently reversing the heart-rate slowing caused by dofetilide. The effects of NS1643 on heart rate in the absence of $I_{Kr}$ blockers, the effects of chronic NS1643 (rather than simply acute administration as performed in this study), and potential interactions with medications, like β-blockers, frequently administered to LQTS patients, remain to be assessed in future work.

In addition to the absolute changes in repolarization delay, variations in spatial dispersion of repolarization may be a very important determinant of TdP occurrence. Changes in other indices, like repolarization dispersion, may contribute to the apparently larger alterations in ectopic-complex frequency vs. QT interval for the first 20 min after drug administration (Supplementary material online, Figure S2), although the lack of statistically significant change in either variable over this time period makes it difficult to draw conclusions. Technical limitations prevented us from measuring reliable indices of repolarization dispersion in the present study, but this will be an important issue to address in future work.

Conclusions

We have shown that acute exposure to an $I_{Kr}$-enhancing drug abbreviates the QT interval and suppresses TdP arrhythmias in two clinically relevant animal models. Patch-clamp studies confirmed the ability of the drug to reverse $I_{Kr}$ suppression in corresponding bradycardia-remodelled and dofetilide-exposed cardiomyocytes. Thus, pharmacological enhancement of $I_{Kr}$ may be an interesting potential approach for patients with arrhythmic conditions caused by impaired cardiac repolarization.

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Supplementary material

Supplementary material is available at Cardiovascular Research online.

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