In vivo mechanisms precipitating torsades de pointes in a canine model of drug-induced long-QT1 syndrome

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Abstract

**Objective:** Congenital loss of function and drug-induced inhibition of the slowly-activating delayed-rectifier K\(^+\) current (\(I_{Ks}\)) cause impaired cardiac repolarization. \(\beta\)-Adrenergic-receptor stimulation contributes to sympathetically-induced torsades de pointes (TdP). An in vivo model of long-QT1 (LQT1) syndrome and TdP in a species with \(I_{Ks}\) characteristics relevant to man is lacking. We investigated the in vivo mechanisms of TdP in a novel canine model of drug-induced LQT1 syndrome.

**Methods:** Adult beagle dogs (\(n=30;\) F/M) were anesthetized with lofentanil (0.075 mg/kg i.v.) and etomidate (1.5 mg/kg/hour). ECGs, left-ventricular (LV) and right-ventricular (RV) monophasic action potentials (MAPs), and intracavitary pressures were recorded simultaneously. Infusion of the \(I_{Ks}\) blocker HMR1556 (0.025–0.050 mg/kg/min) mimicked LQT1, and bolus injections of isoproterenol (1.25–5 \(\mu\)g/kg) reproducibly triggered TdP in 94\% of dogs (defibrillated if necessary).

**Results:** Isoproterenol evoked paradoxical repolarization prolongation during heart rate accelerations. Beat-to-beat variability [QT, LV MAP duration (MAPD\textsubscript{90})] and spatial dispersion of repolarization (T\textsubscript{peak}–T\textsubscript{end} interval, endo-minus-epicardial MAPD\textsubscript{90}, LV-RV MAPD\textsubscript{90}) were significantly increased. Early afterdepolarizations occurred predominantly in the endocardium and not the epicardium. During isoproterenol, secondary systolic contractions (aftercontractions; peak 25±6 mm Hg) arose in the LV (not RV) when TdP ensued. Prevention of TdP by esmolol (1.25 mg/kg), verapamil (0.4 mg/kg) or mexiletine (5 mg/kg) was only successful when repolarization prolongation was contained and aftercontractions remained absent.

**Conclusions:** \(\beta\)-Adrenergic challenges trigger TdP in a reproducible manner in this model of drug-induced LQT1. Paradoxical prolongation and increased temporal and spatial dispersion of repolarization precipitate TdP. Incremental LV systolic aftercontractions precede TdP, suggesting abnormal cellular Ca\(^{2+}\) handling contributes to the arrhythmogenic mechanism.

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**Keywords:** Adrenergic (ant)agonists; Calcium (cellular); Ion channels; Long-QT syndrome; Repolarization

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This article is referred to in the Editorial by L. Fabritz (pages 202–203) in this issue.

1. Introduction

Drug-induced inhibition and congenital loss of function (LQT1) of the slowly-activating delayed-rectifier K\(^+\) current (\(I_{Ks}\)) impair cardiac repolarization. Increased adrenergic tone plays a pivotal role in prolonging and destabilizing ventricular repolarization when \(I_{Ks}\) is reduced, which has been
demonstrated in canine [1,2], rabbit [3] and human myocardium [4]. β-Adrenergic-receptor stimulation contributes to sympathetically-induced torsades de pointes (TdP).

Drug-induced LQT1 and TdP-like polymorphic tachyarrhythmia have been successfully produced in arterially-perfused canine and feline left-ventricular (LV) wedge preparations [1,5]. Induction and prevention or suppression of TdP in these tissue models were related to an increase and decrease, respectively, in the transmural dispersion of repolarization [6,7]. Similarly, in LQT1 patients the heart rate-corrected Tpeak-Tend interval, a surface marker of spatial dispersion of ventricular repolarization [6,7], was found to be increased during exercise stress testing [8] and intravenous (i.v.) infusion of epinephrine [9]. Clinical data indicate further that sympathetic stimulation increases beat-to-beat QT variability in most LQT1 patients [10].

Despite the recognition of intricate relationships between adrenergic stimulation and LQT1-based proarrhythmia in the intact body, an in vivo model of LQT1 in a large species with IKs characteristics relevant to man is lacking. We have now developed such a model in anesthetized dogs using infusion of the selective IKs blocker HMR1556 to mimic LQT1, and β-adrenergic challenges with isoproterenol to trigger TdP. ECGs and monophasic action potentials (MAPs) were simultaneously recorded to examine the relationship between putative electrophysiological markers and the actual induction and treatment of TdP. These studies revealed inappropriate repolarization prolongation and dispersion during β-adrenergic-evoked heart rate accelerations, often followed by arrhythmia. Intracardiac pressure recordings yielded the novel insight that incremental systolic after-contractions of the LV immediately preceded the TdP. These data, and the results from experiments using drugs that affect cellular Ca2+ load, suggest abnormal Ca2+ handling as a contributing arrhythmogenic mechanism.

2. Methods

This investigation conformed with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.1. In vivo experiments

Thirty adult beagle dogs (age ≥12 months; F/M; body weight 11.1±0.3 kg) were used in this study. General anesthesia was induced by isoflurane (0.075 mg/kg body weight i.v.), scopolamine (0.0015 mg/kg), succinylcholine (1.0 mg/kg), hourly slow injections of fentanyl (0.025 mg/kg i.v.), and continuous infusion of etomidate (1.5 mg/kg/hour) [11]. During anesthesia the heart rate was similar to that in conscious beagle dogs (n=17) of the same population (80±5 bpm versus 89±3 bpm, respectively; P=NS). Based on our own results and literature data the measured plasma concentrations of any of these anesthetics are not expected to significantly affect myocardial K+ currents, notably the rapidly-activating delayed-rectifier K+ current (IK).

Dogs were ventilated with 30% oxygen in pressurized air to normocapnia. The body temperature was kept at 37 °C with a heated water mattress. ECG standard lead II was continuously recorded and the QT interval (QT; ms) measured from the onset of the QRS to the final end of the T wave. LV and right-ventricular (RV) intracavitary pressures were recorded with high-fidelity catheter-tip microcomputers ( Gaeltec Ltd, Dunvegan, UK and Millar Instruments Inc, Houston, TX). Under fluoroscopic guidance, MAP catheters (Boston Scientific-EP Technologies, San Jose, CA) were placed simultaneously at the endocardium of the LV and RV, near the apical septum. In 6 open-chest dogs, MAP catheters were positioned oppositely at the endocardium and epicardium of the LV free wall. MAP duration was measured at 90% repolarization (LV MAPD90 and RV MAPD90, ms). Temporal dispersion of repolarization, otherwise named beat-to-beat variability of repolarization duration (BVR) [12] or instability (STI) [11] for 30 (or a minimum of 10) consecutive beats was determined from Poincaré plots as the mean orthogonal distance from the plot coordinates to the line y=x.

HMR1556, a selective blocker of KCNQ1 [13], was administered to mimic LQT1 syndrome. In separate patch-clamp experiments on cultured mammalian cells stably expressing human KCNQ1/KCNE1 or KCNH2 channels, we found that HMR1556 dose-dependently inhibited KCNQ1/KCNE1 current with an IC50 of 74 nmol/L. In contrast, concentrations up to 10 μmol/L (the limit of solubility) inhibited KCNH2 current to a maximum of 10%. These results are in line with the pronounced selectivity of HMR1556 for IKs in native myocytes [14].

HMR1556 (dissolved in 20% HP-β-cyclodextrin) was infused i.v. in the dogs, initially at a rate of 0.025 mg/kg/min.

### Table 1

<table>
<thead>
<tr>
<th>Dose-dependent induction of sustained TdP by i.v. HMR1556 and isoproterenol</th>
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<tbody>
<tr>
<td>Cumulative number of TdP-inducible dogs</td>
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<td>---------------------------------------------</td>
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<tr>
<td>HMR1556, low infusion rate 0.025 mg/kg/min, maximal cumulative dose 0.75 mg/kg</td>
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<td>2/18</td>
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<td>5/18</td>
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<td>6/18</td>
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<tr>
<td>HMR1556, medium infusion rate 0.050 mg/kg/min, maximal cumulative dose 2.25 mg/kg</td>
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</table>

Data from 18 dogs.
for 30 min and followed (if necessary) by infusions at 0.05 mg/kg/min and 0.1 mg/kg/min. At regular time intervals, boluses of isoproterenol (1.25, 2.5 or 5 μg/kg) were injected to induce TdP. External electrical cardioversion was applied to terminate sustained TdP or its deterioration into ventricular fibrillation, if induced by isoproterenol. The effects of antiarrhythmic drugs esmolol (1.25 mg/kg i.v.), verapamil (0.4 mg/kg i.v.) and mexiletine (5 mg/kg i.v.) on the inducibility of TdP were also examined.

Arterial blood samples were taken regularly for measurements of HMR1556 concentrations in plasma using liquid chromatography–mass spectroscopy. HMR1556 (M_w = 411.4 g/mol) was a kind gift from Dr. Heinz Gögelein, Sanofi-Aventis Germany GmbH, Frankfurt, Germany. Lofentanil, scopolamine, fentanyl, and etomidate were obtained from Janssen Pharmaceutica, Beerse, Belgium, and succinylcholine from Christiaens N.V., Brussels, Belgium.

2.2. Data analysis

Group data are expressed as mean±SEM. Intergroup comparisons were made with ANOVA Dunnett’s test on repeated measures. A two-tailed P<0.05 was considered statistically significant. Fisher’s probability test was used to evaluate the differences in the incidence of early afterdepolarizations (EADs), aftercontractions or TdP between TdP-inducible and non-inducible conditions.

3. Results

3.1. Reproducible induction of TdP by isoproterenol in HMR1556-infused dogs

Of the total of 30 dogs, 18 were used in a dose-finding study to determine which combinations of HMR1556 and isoproterenol were torsadogenic in the majority of animals. Cumulative and separate data are shown in Table 1. After infusion of 1.75 mg/kg HMR1556 (the result of 30 min at 0.025 mg/kg/min+20 min at 0.050 mg/kg/min; Table 1, Medium Infusion Rate), a bolus of 2.5 μg/kg isoproterenol induced TdP in 17 of 18 animals (94%).

The reproducibility of TdP induction was examined in all 18 dogs. TdP was produced and after spontaneous cardioversion (nonsustained TdP) or defibrillation (sustained TdP) to sinus rhythm and a recovery period of 15 min, the same doses of HMR1556 and isoproterenol that any one dog received consistently induced new episodes of TdP, as exemplified in Fig. 1. TdP could thus be triggered at least 3 times in individual dogs. Plasma concentrations of HMR1556 were similar at first and second episodes of TdP.

The 12 other dogs were instrumented with LV and RV catheters to focus in detail on electrophysiological and hemodynamic responses, and markers for TdP. In these 12 dogs, all showing TdP, we subsequently tested the effects of esmolol, verapamil and mexiletine in the same experiment. The results are presented below.

![Fig. 1. A, QT prolongation (hatched bar) and broad-based T waves by HMR1556 (0.05 mg/kg/min) in an anesthetized dog. B, Reproducible induction of TdP by isoproterenol in drug-induced LQT1 syndrome. Simultaneous ECG (lead II) and LVP at baseline, during infusion of 0.05 mg/kg/min HMR1556 (t=14 min) and upon washout of HMR1556 (t=40 min). Boluses of isoproterenol caused heart rate accelerations, ventricular ectopic beats and episodes of nonsustained (t=16 min; 1.25 μg/kg isoproterenol) and sustained TdP (t=22 min; 2.5 μg/kg). Numbers at ECG indicate RR intervals (above) and QT times (below) in ms. Arrows at t=22 min (LVP), crescendo aftercontractions. Vertical and horizontal scale bars apply to all panels.](https://academic.oup.com/cardiovascres/article-abstract/76/2/247/269209)
3.2. Prolongation of ventricular repolarization by HMR1556

Table 2 (middle column) shows electrophysiological and hemodynamic responses to HMR1556 alone. After a loading period of 30 min at 0.025 mg/kg/min, this infusion rate was continued in 3/12 animals and doubled in the other 9 (0.05 mg/kg/min) to reach similar plasma concentrations (see also Table 1). P-wave and QRS-complex morphology, and PQ (110±2 ms at baseline) and QRS intervals (45±2 ms) did not change during HMR1556. QT prolonged by 27% and heart rate-corrected QT time (QTcVdW) [15] by 25% (both P<0.05). T waves were broad-based and asymmetrical, and had an unaltered polarity (Fig. 1A). Tpeak–Tend interval was significantly augmented. LV MAPD90 increased more than RV MAPD90, thus amplifying the physiological interventricular dispersion of repolarization (LV-RVMAPD90). Parameters of temporal dispersion (LV BVR, QT BVR and QT STI) did not change significantly. EADs were not observed in either ventricle in the presence of HMR1556 alone, nor did TdP occur.

Peak systolic LV pressure (LVPsystolic) rose by 16% (P<0.05; Table 2) during HMR1556 and this effect was attended by an increased maximal rate of rise of LVP (LV dP/}

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Electrophysiological and hemodynamic responses to HMR1556 and HMR1556 plus isoproterenol in TdP-inducible dogs</th>
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<tbody>
<tr>
<td>Heart rate, bpm</td>
<td>Baseline</td>
</tr>
<tr>
<td>QT, ms</td>
<td>78±4</td>
</tr>
<tr>
<td>QTcVdW, ms</td>
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<tr>
<td>LV MAPD90, ms</td>
<td>224±8</td>
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<tr>
<td>RV MAPD90, ms</td>
<td>211±7</td>
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<tr>
<td>LV-RVMAPD90, ms</td>
<td>14±2</td>
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<tr>
<td>LV MAPDendocardium</td>
<td>29±5</td>
</tr>
<tr>
<td>LV MAPDendocardium</td>
<td>33±4</td>
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<tr>
<td>LV BVR, ms</td>
<td>1.7±0.2</td>
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<tr>
<td>QT BVR, ms</td>
<td>1.4±0.3</td>
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<tr>
<td>QT STI, ms</td>
<td>1.4±0.5</td>
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<tr>
<td>LVPsystolic, mm Hg</td>
<td>135±6</td>
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<tr>
<td>LVPendocardium, mm Hg</td>
<td>14±1</td>
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<tr>
<td>LV dP/dtmax, mm Hg/s</td>
<td>3419±206</td>
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<tr>
<td>τrelaxation, ms</td>
<td>22±1</td>
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<tr>
<td>Cl to peak LVP, ms</td>
<td>181±7</td>
</tr>
<tr>
<td>LVPduration, ms</td>
<td>279±5</td>
</tr>
<tr>
<td>Amplitude of aftercontraction, mm Hg</td>
<td>25±6</td>
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<tr>
<td>Duration of aftercontraction, ms</td>
<td>125±9</td>
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<tr>
<td>Cl to start aftercontraction, ms</td>
<td>240±7</td>
</tr>
<tr>
<td>Cl to peak aftercontraction, ms</td>
<td>304±12</td>
</tr>
<tr>
<td>Cl to start EAD, ms</td>
<td>263±9</td>
</tr>
<tr>
<td>Cl to peak EAD, ms</td>
<td>295±12</td>
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</tbody>
</table>

Data from 12 dogs. QT, VdW, heart rate-corrected QT time according to Van de Water’s formula [15]; LV, left ventricular; RV, right ventricular; MAPD90, monophasic-action-potential duration measured at 90% repolarization; Tpeak–Tend interval, spatial dispersion of ventricular repolarization; BVR, beat-to-beat variability of repolarization [12]; STI, short-term instability of ventricular repolarization [11]; LVP, LV pressure; Cl, coupling interval; EAD, early afterdepolarization; *, P<0.05 versus baseline; †, P<0.05 versus HMR1556.

3.3. Markers for TdP in drug-induced LQT1 syndrome

In the example of Fig. 1B, infusion of HMR1556 for 15 min, followed by a bolus injection of isoproterenol, caused an acceleration of heart rate and, after about 4 s, ventricular ectopic beats and nonsustained TdP (t=16 min). Six minutes later, another bolus of isoproterenol caused sustained TdP that was terminated by electrical cardioversion (t=22 min). One more episode of sustained TdP was induced in this experiment (not shown) after which sinus rhythm was regained (t=40 min). LVP recordings demonstrated hemodynamic collapse during TdP and full recovery upon electrical cardioversion.

Isoproterenol evoked paradoxical repolarization prolongation during heart rate accelerations in experiments in which TdP was subsequently induced. It took on average 4.2±0.2 s (n=12 dogs) from the first measurable effects of isoproterenol until the initiation of TdP. Beat-by-beat changes (depicted as −6 to 0) of QT and RR intervals in these final moments before arrhythmia are shown in Fig. 2, left panel. RR shortened progressively and did not exhibit short–long–short sequences with long pauses. R-on-T superpositions (arrow, Fig. 2, left panel) occurred at a RR interval of 383±12 ms and a QT of 385±15 ms, and were often caused or followed by TdP-inciting ventricular ectopic beats. In experiments in which TdP could not be induced, isoproterenol evoked QT shortening rather than prolongation (Fig. 2, middle panel). Under these circumstances HMR1556 plasma concentrations were lower than in TdP-inducible experiments (776±44 ng/mL versus 1393±105 ng/mL, respectively; P<0.05), as reflected by a less prolonged QT time (compare beat −6 in left and middle panels of Fig. 2). β-Adrenergic responses under baseline anesthetized conditions (no HMR1556) are shown in Fig. 2, right panel. Isoproterenol alone did not induce EADs or TdP.

LV BVR, QT BVR and QT STI were markedly exaggerated by isoproterenol when TdP ensued (Table 2). Likewise, parameters of spatial dispersion of repolarization were significantly increased: Tpeak–Tend interval by 32% and LV-RVMAPD90 by 46% (both P<0.05 versus HMR1556 alone). Fig. 3 demonstrates that the prolongation of repolarization by isoproterenol was predominantly observed in the endocardium, not epicardium, causing significant amplification of local dispersion of repolarization (LV MAPDendocardium), sometimes even up to >100 ms in single beats, followed by initiation of TdP. Also, EADs occurred more frequently in the LV endocardium (5/6 dogs) than epicardium (2/6 dogs), and more frequently in the LV endocardium than RV endocardium (1/6 dogs). Both late EADs (generated during phase 3 of the MAP) and early EADs (plateau phase) were encountered (Fig. 4). Based on ECG and MAPs, TdP originated almost exclusively in the LV, not RV.
LVPsystolic peak pressures were not elevated during isoproterenol compared to HMR1556 alone (Table 2). However, both LV $dP/dt_{\text{max}}$ and $\tau_{\text{relaxation}}$ were significantly changed, by +66% and −18% respectively. As a result, LVPduration shortened significantly from 286±7 ms to 190±7 ms, despite the prolongation of repolarization. The coupling interval (CI)

Fig. 2. Left panel, paradoxical QT prolongation during isoproterenol-evoked heart rate accelerations in HMR1556 experiments in which TdP was induced. Shown are beat-by-beat changes of QT and RR intervals (mean ± SEM; $n=10$ dogs) in final moments before TdP. RR shortened progressively and did not exhibit short–long–short sequences. Arrow indicates R-on-T superposition. Middle panel, QT and RR intervals in experiments in which TdP could not be induced ($n=5$ dogs). All 5 animals were TdP-inducible at higher doses of HMR1556 and/or isoproterenol. Right panel, $\beta$-adrenergic responses under baseline anesthetized conditions (no HMR1556; $n=5$ dogs). Horizontal dotted line spanning 3 panels arbitrarily drawn for comparison. * $P<0.05$ for RRbeat versus RRbeat−6, † $P<0.05$ for QTbeat versus QTbeat−6, ‡ $P<0.05$ for QTbeat−6 of left versus middle panel.

Fig. 3. Repolarization prolongation and instability by isoproterenol occur predominantly in LV endocardium, not epicardium. Simultaneous ECG, LV MAPepi, LV MAPendo and LVP from an anesthetized open-chest dog at baseline, during 0.05 mg/kg/min HMR1556 ($t=15$ min) and upon bolus of 2.5 μg/kg isoproterenol ($t=16$ min). Numbers at ECG indicate RR intervals (above) and QT times (below) in ms. Numbers below MAP recordings, MAPD90 in ms. Arrows at LVP, aftercontractions.
from MAP upstroke to peak LVPsystolic also shortened. In this discordance of contractile and electrical effects, de-novo secondary systolic contractions (aftercontractions) emerged in the LV (not RV) when TdP ensued (see arrows at LVP recordings in Figs. 1B, 3, 4 and 5). These aftercontractions commenced well before the completion of LV repolarization (240±7 ms versus 292±13 ms of LV MAPD90; \( P<0.05 \); Table 2), and even before the onset of concomitant late EADs (240±7 ms versus 263±9 ms; \( P<0.05 \)). Aftercontraction amplitudes grew beat-by-beat, reaching peak pressures of 25±6 mm Hg (17% of primary “normal” contractions) just prior to TdP. These aftercontractions (more so than EADs) appeared to coincide with delayed activity in the nadir of the T wave (Fig. 4).

3.4. Effects of antiarrhythmic drugs

In the same 12 dogs, we subsequently examined the effects of esmolol, verapamil and mexiletine. Per agent 4 animals underwent the testing. Thus, a given dog did not receive more than one antiarrhythmic treatment. Fig. 5 demonstrates that a bolus of 1.25 mg/kg of esmolol prevented the induction of TdP (\( t=46 \) min), whereas the arrhythmia had been inducible and sustained before (\( t=16 \) min). Three other dogs showed similar findings (Fig. 6). Interestingly, the paradoxical prolongation of repolarization and the crescendo aftercontractions that preceded TdP before esmolol remained absent after administration of the β-blocker.

We also tested the effects of verapamil (0.4 mg/kg) and mexiletine (5 mg/kg). Similar to esmolol, infusion of verapamil completely prevented the occurrence of TdP in all 4 animals, and no aftercontractions were observed (\( P<0.05 \)). In 2/4 dogs, however, EADs were still observed. Mexiletine prevented TdP, aftercontractions and EADs in only 1 of 4 animals tested (Fig. 6, arrow). In the non-protected dogs aftercontractions and EADs were still present.

![Fig. 4. Electrical and contractile instability by isoproterenol occur predominantly in the LV, not RV. Simultaneous ECG, LVP, LV MAPendo, RVP and RV MAPendo from a closed-chest dog. Left panel, superposition of beat during HMR1556 (black; pre-ISO) and final 4 beats preceding TdP (colors; beats -3 to 0), as well as inciting ventricular ectopic beat, during isoproterenol. Dashed vertical line: timing of events compared to initiation of LV aftercontractions. Right, same beats put consecutively as recorded. Note the increasing T-wave alterations, beat-by-beat increment of aftercontractions in LVP (arrows), and the presence of phase-2 (early) and phase-3 (late) EADs on LV MAP.](https://academic.oup.com/cardiovascres/article-abstract/76/2/247/269209)
At 20 min after infusion of esmolol, verapamil and mexiletine, TdP was reinducible after a new bolus of isoproterenol in 11/12 dogs. In all cases the arrhythmia was preceded by the reappearance of LV aftercontractions (Fig. 6).

### 4. Discussion

#### 4.1. An in vivo canine model of drug-induced LQT1 syndrome and reproducible TdP

We have developed a new model of drug-induced LQT1 syndrome in anesthetized dogs with normal sinus rhythm. In this model, β-adrenergic challenges with isoproterenol trigger TdP in a consistent and reproducible manner. LQT1 is mimicked by the i.v. infusion of HMR1556, which causes dose-dependent prolongation of ventricular repolarization consistent with previous data in conscious dogs after oral administrations [2]. Spatial dispersion of repolarization ($T_{\text{peak}} - T_{\text{end}}$ interval, LV $\text{MAPD}_{\text{endo-epi}}$, LV-RVMAPD$_{90}$) is significantly increased by this $I_{\text{ks}}$ blockade. In the absence of additional β-adrenergic-receptor stimulation no TdP is triggered. Similarly, in canine [1] and feline [5] LV wedge preparations TdP-like arrhythmia was also not inducible after administration of the $I_{\text{ks}}$ blocker chromanol 293B alone, whereas the addition of isoproterenol predisposed to the arrhythmia by increasing transmural dispersion of repolarization [1]. Therapeutic concentrations of propranolol prevented these proarrhythmic effects. Mexiletine diminished chromanol-293B-accentuated transmural dispersion of repolarization and prevented isoproterenol-provoked TdP [1].

The specific anesthetics chosen for this study have only limited influence on β-adrenergic responsiveness and baroreflex sensitivity, and thus are preferred in studies on β-adrenergic-induced TdP over anesthetic regimes that cause autonomic blockade. The availability of an in vivo canine model of LQT1, as presently introduced, is of great value in addition to tissue models because it enables studies of the intricate relationships between the autonomic nervous system and proarrhythmia in the intact body, as well as the assessment of Ca$^{2+}$- and load-dependent influences on the occurrence of TdP in the beating heart. The induction of TdP neither requires programmed electrical stimulation nor the predisposition of electrical remodeling as in other models.

In our experiments in which sustained TdP was induced, plasma levels of HMR1556 averaged ∼1400 ng/mL (∼3.4 μmol/L). Taking into account that 75% of the drug is bound to plasma proteins [16], these data indicate a free HMR1556 fraction of ∼850 nmol/L. Based on previous

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**Diagram 5. β-Blockade with esmolol prevents induction of TdP by isoproterenol.** Simultaneous ECG (lead II), LV MAP$_{\text{endo}}$ and LVP recordings at baseline ($t=0$ min), during infusion of 0.025 mg/kg/min HMR1556 ($t=14$ min) and upon 2.5 μg/kg isoproterenol causing TdP ($t=16$ min). After defibrillation, the addition of esmolol (1.25 mg/kg) to continued infusion of HMR1556 ($t=45$ min) prevented reinduction of TdP ($t=46$ min). Numbers indicate RR intervals (above) and QT times (below) in ms. Arrows at LVP, aftercontractions.
results in canine ventricular myocytes such a concentration is expected to block $I_{Ks}$ almost completely [2,14], while exerting minimal, if any, effects on $I_{Kr}$ and other ion currents [14].

4.2. Mechanisms precipitating TdP

A consistent feature foreshadowing TdP in drug-induced LQT1 syndrome is repolarization prolongation during $\beta$-adrenergic-evoked heart rate acceleration. This is likely caused by the failure of action-potential shortening, due to the inhibition of $\beta$-adrenergic-sensitive $I_{Ks}$, and in addition prolongation by the overriding of (Ca$^{2+}$-dependent) inward currents. Repolarization prolongation and EAD generation occur predominantly in the LV, not RV (Fig. 4), and are more pronounced in the LV endocardium than epicardium (Fig. 3). This LV predominance appears to be unique for our LQT1 model; it is not a known feature of arrhythmogenesis in other LQT conditions.

The combination of prolonged repolarization and shortened LVP$_{systolic}$ favors the emergence of LV systolic aftercontractions that are only observed when TdP ensues. Interestingly, these contractile eruptions commence tens of ms earlier than late EADs in the same beat and occur also in the absence of EADs. This could mean that the aftercontractions may be initiated by local afterdepolarizations not recorded by the MAP catheters. Alternatively, assuming that EADs in MAP signals are representative of transmembrane EADs, these findings suggest that the generation of systolic aftercontractions is not necessarily dependent on Ca$^{2+}$-induced Ca$^{2+}$ release through ($\beta$-adrenergic-enhanced) window L-type Ca$^{2+}$ current, but can also result from regenerative spontaneous Ca$^{2+}$ release from the sarcoplasmic reticulum in the setting of Ca$^{2+}$ overload. The latter mechanism has been demonstrated for isoproterenol-induced aftercontractions in canine ventricular myocytes [17]. Finally, we have to consider that aftercontractions may actually cause the EADs through mechano-electrical influences in the beating heart.

The present results demonstrate that in vivo $I_{Ks}$ blockade does not significantly exaggerate repolarization instability unless $\beta$-adrenergic-receptor stimulation is added. This implies that an $I_{Ks}$-depleted repolarization may often be disguised as latent instability and that relatively mild stimuli
can have profound proarrhythmic consequences. β-Adrenergic provocation may exacerbate spatial and temporal dispersion of repolarization, favoring reentry under these conditions. EAD generation further fuels this proarrhythmic substrate. Incremental LV aftercontractions likely predispose to LV ectopic activity through triggered activity. Ventricular ectopic beats infringing on the reentrant substrate ignite TdP.

4.3. Clinical implications

Our new canine model displays many of the electrophysiological characteristics of the human congenital LQT1 syndrome. In LQT1 patients, ECG markers of spatial and temporal dispersion of ventricular repolarization are amplified during provocation testing with epinephrine [9,10]. Moreover, epinephrine prolongs the LV MAPD90 and evokes temporal dispersion of ventricular repolarization are ampli- 

sic syndrome. In LQT1 patients, ECG markers of spatial and physiological characteristics of the human congenital LQT1 

4.4. Limitations

The dogs used in these experiments do not carry the genetic fingerprints of human congenital LQT1 syndrome and the use of HM1556 will not cover all of the biophysical changes caused by genetic KCNQ1 mutations. However, our new canine model may be preferable over transgenic rodent LQT1 models because the cardiac electrophysiological properties of dogs are quite similar to those of humans and very different from those of mouse, rat and guinea pig. This includes the kinetics of IKs and IKr, which has major implications for the mechanisms of TdP.

4.5. Conclusions

The availability of a new model of drug-induced LQT1 syndrome in anesthetized dogs offers great opportunities to improve mechanistic understanding and adequate therapy of sympathetically-induced TdP. In this model, β-adrenergic provocation with isoproterenol triggers TdP in a consistent and reproducible manner. Electrophysiological recordings indicate that exaggerated regional and temporal dispersion of repolarization, as well as EADs, originate predominantly from the LV (sub)endocardium. TdP is most often triggered by focal activity in the LV. Among the multiple markers we have examined, paradoxical LV repolarization prolongation and incremental systolic aftercontractions with R-on-T superpositions during β-adrenergic heart rate accelerations are the most predictive of impending TdP. Prevention of TdP by esmolol, verapamil or mexiletine is only successful when this repolarization prolongation is contained and after- contractions remain absent.

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