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RESEARCH PAPER

Slow delayed rectifier K^+ current block by HMR 1556 increases dispersion of repolarization and promotes Torsades de Pointes in rabbit ventricles

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Background and purpose: The slow delayed rectifier K^+ current (I_{Ks}) contributes to ventricular repolarization when the action potential (AP) is prolonged. I_{Ks} block during drug-induced AP prolongation may promote Torsades de Pointes (TdP), but whether this is due to additional AP prolongation is uncertain.
Experimental approach: In bradycardic perfused rabbit ventricles, the incidence of spontaneous TdP, monophasic AP duration at 90% repolarization ($MAPD_{90}$) and ECG interval between the peak and the end of T wave ($T_{peak-end}$) (index of dispersion of repolarization) were measured after the administration of veratridine (125 nM, slows Na^+ channel inactivation), dofetilide (7.5 or 10 nM, a rapid delayed rectifier blocker) and HMR 1556 (HMR, 100 nM, an I_{Ks} blocker), alone or in combinations ($n=6$ each).
Key results: HMR did not prolong $MAPD_{90}$, whereas veratridine or 7.5 nM dofetilide prolonged $MAPD_{90}$ ($P<0.01$) without inducing TdP. Veratridine + 7.5 nM dofetilide additively prolonged $MAPD_{90}$ ($P<0.05$), induced 4 ± 6 TdP per heart and prolonged $T_{peak-end}$ by 12 ± 10 ms. Subsequent addition of HMR did not further prolonged $MAPD_{90}$, but increased the number of TdP to 22 ± 18 per heart and increased $T_{peak-end}$ by 39 ± 21 ms ($P<0.05$). Increasing dofetilide concentration from 7.5 to 10 nM (added to veratridine) produced a longer $MAPD_{90}$, but fewer TdP (5 ± 5 per heart) and less $T_{peak-end}$ prolongation (17 ± 8 ms) compared to the veratridine + 7.5 nM dofetilide + HMR group ($P<0.05$).
Conclusions and implications: Adding I_{Ks} block markedly increases TdP incidence in hearts predisposed to TdP development by increasing the dispersion of repolarization, but without additional AP prolongation.
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Keywords: Torsades de Pointes; action potential prolongation; dispersion of repolarization; I_{Ks}; HMR 1556; dofetilide; veratridine; perfused rabbit ventricles

Abbreviations: AP, action potential; APD, AP duration; CL, cycle length; HMR, HMR 1556; I_K, delayed rectifier K^+ current; I_{Kr}, the rapid component of I_K; I_{Ks}, the slow component of I_K; I_{Na}, sodium current; LV, left ventricular; LVP, LV pressure; MAP, monophasic AP; $MAPD_{90}$, MAP duration at 90% repolarization; Torsades de Pointes, TdP; $T_{peak-end}$, the interval between the peak and the end of T wave

Introduction

The balance of inward and outward ionic currents of the heart is crucial in forming a normal cardiac action potential (AP). The delayed rectifier K^+ current (I_K) is the key outward current for cardiac repolarization and is composed of rapid (I_{Kr}) and slow (I_{Ks}) components (Sanguinetti and Jurkiewicz, 1990). The presence of both components is postulated to provide some redundancy or 'repolarization reserve', thereby preventing excessive prolongation of repolarization (Roden, 2006).

Torsades de Pointes (TdP) is a serious polymorphic ventricular tachycardia caused by reductions in repolarizing currents or increases in depolarizing currents, resulting in prolonged AP duration (APD). Reductions in I_{Kr} owing to genetic mutations can lead to congenital long QT-2 syndrome and TdP, and this subtype accounts for 39% of the total long QT syndrome cases (Napolitano *et al.*, 2005). Nevertheless, I_{Kr} blockers, which prolong cardiac refractoriness, are used clinically to treat atrial and ventricular tachyarrhythmias, but they can sometimes induce TdP. I_{Kr} blockade is also responsible for the increased incidence of

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TdP and unexpected death associated with non-cardiac drugs, such as certain antihistamines and antimicrobials (Woosley *et al*., 1993; Bischoff *et al*., 2000; Heist and Ruskin, 2005).

Unlike I_{Kr}, I_{Ks} contributes to ventricular repolarization as a function of heart rate and the extent of β-adrenoceptor stimulation (Marx *et al*., 2002; Silva and Rudy, 2005). I_{Ks} blockade produces little AP prolongation in rabbit, canine and human ventricles (Biliczki *et al*., 2002; Jost *et al*., 2005; So *et al*., 2006), unless it is in the presence of catecholamines (Jost *et al*., 2005; So *et al*., 2007). Moreover, I_{Ks} acts as a 'repolarization reserve' (Roden, 2006), and its blockade further prolongs the AP when repolarizing currents are reduced or when depolarizing currents are increased (Biliczki *et al*., 2002; Nakashima *et al*., 2004; Jost *et al*., 2005; So *et al*., 2005, 2006). Long QT-1 syndrome caused by genetic mutation of I_{Ks} is the most common subtype of long QT syndrome (49% of the total long QT syndrome cases) (Napolitano *et al*., 2005). Although long QT-1 patients usually develop TdP owing to inability to shorten the QT interval during β-adrenoceptor stimulation (for example, during exercise), previous studies have shown that adding I_{Ks} block to I_{Kr} block increased the incidence of TdP (Lengyel *et al*., 2007; Michael *et al*., 2007), indicating that I_{Ks} may provide 'repolarization reserve' against drug-induced TdP. It has been proposed that variability in I_{Ks} expression among individuals may lead to differences in the extent of 'repolarization reserve', which may not be reflected by the resting QT interval (Roden, 2006). This may explain the variable susceptibility to develop drug-induced TdP in individuals with similar baseline QT interval.

Although previous findings suggest that I_{Ks} block can be proarrhythmic in the presence of drug-induced AP prolongation (Lengyel *et al*., 2007; Michael *et al*., 2007), it is unclear whether it was caused by additional QT prolongation or other arrhythmogenic mechanisms, such as increase in dispersion of ventricular repolarization. I_{Ks} is heterogeneously expressed across the ventricular layers, with the mid-myocardium expressing the least I_{Ks} compared with the epicardium and endocardium (Liu and Antzelevitch, 1995). Adding I_{Ks} block to I_{Kr} block has been shown to increase dispersion of ventricular repolarization, a condition that promotes re-entrant tachyarrhythmias (Burashnikov and Antzelevitch, 2002).

To investigate the mechanisms by which I_{Ks} blockade promote TdP, we have established a proarrhythmic perfused rabbit heart model through the combination of I_{Kr} block and slowing of sodium channel (I_{Na}) inactivation, in the presence or absence of I_{Ks} block. This study investigated whether adding I_{Ks} block to drug-induced AP prolongation promotes TdP, and whether it is owing to additional AP prolongation or increase in dispersion of ventricular repolarization.

Methods

Langendorff perfused rabbit hearts

All animal procedures were in accordance with the UK Animals Act 1986 for scientific procedures and approved by the Animal Care Committee of St Michael's Hospital. All animals were maintained with standard food, water and light/dark cycles according to the ethical guidelines. Hearts isolated from 42 New Zealand White rabbits (3.0–4.0 kg, male) were perfused at constant pressure using the Langendorff method as previously described (So *et al*., 2006).

Electrophysiological studies

To facilitate the development of drug-induced TdP at slow ventricular rates, the atrioventricular node was ablated by crushing with forceps. To determine the rate-corrected monophasic AP (MAP) duration, the ventricles were paced at a cycle length (CL) of 500 ms using a 7F quadripolar contact Ag-AgCl MAP catheter (EP Technologies Inc., Sunnyvale, CA, USA) and a programmable stimulator (Medtronic 5325, Minneapolis, MN, USA) with 2 ms pulse width and twice the diastolic threshold. The MAP duration at 90% repolarization ($MAPD_{90}$) and QT interval were measured after a 20 s conditioning train at CL of 500 ms. As drug-induced changes in conduction might affect the inducibility of proarrhythmic events, QRS duration at pacing CL of 500 ms was measured from the left ventricular (LV) epicardial bipolar electrogram and did not change in any group.

Spontaneous TdP or R on T premature beats were measured during ventricular escape rhythm without pacing for 10 min. Spontaneous TdP was defined as at least four consecutive beats of polymorphic QRS complexes (including the first beat following a pause), with an initiating beat falling on the T wave of the preceding beat after a pause ('pause-dependent' initiation). Spontaneous 'R on T premature beats' were defined as any premature beats whose onset occurred before the MAP of the preceding beat had recovered back to the baseline, but did not lead to the initiation of spontaneous TdP.

An increase in dispersion of repolarization is a known risk factor for TdP development. The interval between the peak and the end of the T wave ($T_{peak-end}$) is a proposed surrogate measure of dispersion of ventricular repolarization (Yan and Antzelevitch, 1998). $T_{peak-end}$ was measured from the unipolar LV electrogram during pacing at CL of 500 ms. Instability of ventricular repolarization is another predictor of TdP development, which can be reflected by beat-to-beat variability in AP (Hondeghem *et al*., 2001; Thomsen *et al*., 2006). The standard deviation of 10 consecutive $MAPD_{90}$ during pacing at CL of 500 ms was measured as an index of beat-to-beat variability of ventricular repolarization.

Drug administration

Untreated control experiments were performed to assess the electrophysiological stability of the preparation over time ($n = 6$). The effects of a selective I_{Ks} blocker HMR 1556 (HMR, Sanofi-Aventis, Frankfurt am Main, Germany), veratridine (Sigma-Aldrich, St Louis, MO, USA), which slows I_{Na} inactivation or an I_{Kr} blocker dofetilide (Pfizer Canada Inc, Quebec, Canada) were studied alone or in combination. The single drug groups included HMR (100 nM), veratridine (125 nM) and dofetilide (7.5 nM) ($n = 6$ each). The combined drug groups included 125 nM veratridine + 7.5 nM dofetilide,

125 nM veratridine + 7.5 nM dofetilide + 100 nM HMR and 125 nM veratridine + 10 nM dofetilide ($n = 6$ each). After baseline measurements were complete, the above drugs and their combinations were administered to the circulating perfusate, and a 15 min equilibration time was allowed before any measurements. HMR was dissolved in dimethylsulphoxide as a stock solution of 16 mM. Veratridine was dissolved in dimethylsulphoxide as a stock solution of 24.7 mM. The highest dimethylsulphoxide concentration in the perfusate was <0.001%. Dofetilide was dissolved in saline as a stock solution of 22.7 μM.

Coronary flow rate and LV systolic pressure measurement

As any drug-induced alterations in cardiac hemodynamics and contractility might affect the inducibility of proarrhythmic events, coronary flow rate and LV systolic pressure were continuously monitored. Coronary flow rate ($mL\,min^{-1}$) was determined 15 min after each drug administration, by measuring the volume of perfusate flowing out of the heart per minute. The LV systolic pressure was measured as the peak pressure generated when the ventricles were paced at CL of 500 ms. There were no differences in the coronary flow rate and LV systolic pressure between the untreated control group and all the treatment groups.

Data analysis and statistics

The normality of data was tested using the Kolmogorov–Smirnov test. As the data passed the normality test, parametric tests were used and the results are expressed as mean ± s.d. Student's unpaired *t*-test was used to compare results of two different groups. Multiple group comparisons were performed by one-way ANOVA. Bonferroni *post hoc* corrections were performed when *P*-values were <0.05, which was considered statistically significant. Correlation analysis was performed using the Pearson's correlation. All statistical analyses and curve-fitting were performed using GraphPad Prism version 4.01 for Windows (GraphPad Software, San Diego, CA, USA).

Results

To investigate the protective function of I_{Ks} in preventing TdP development when AP is substantially prolonged, we perfused rabbit hearts with veratridine (which slows I_{Na} inactivation), the I_{Kr} blocker dofetilide and the I_{Ks} blocker HMR, alone or in combination. Representative MAP tracings obtained in untreated control experiments and after the individual administration of 100 nM HMR, 125 nM veratridine or 7.5 nM dofetilide are shown in Figure 1a. Control hearts had no change in $MAPD_{90}$ over time ($P = 0.25$, $n = 6$). The I_{Ks} blocker HMR alone also had no effect on $MAPD_{90}$ ($P = 0.63$, $n = 6$, Figure 1b). In contrast, administration of veratridine or dofetilide alone significantly prolonged $MAPD_{90}$ ($P < 0.01$, $n = 6$, Figure 1b). No proarrhythmic events were observed when HMR, veratridine or dofetilide were administered alone.

As our previous studies indicate that adding I_{Ks} block to either dofetilide or veratridine produced a modest APD prolongation without TdP induction in perfused rabbit hearts (So *et al*., 2005, 2006), we combined veratridine and dofetilide in an attempt to achieve greater APD prolongation. As expected, veratridine (125 nM) and dofetilide

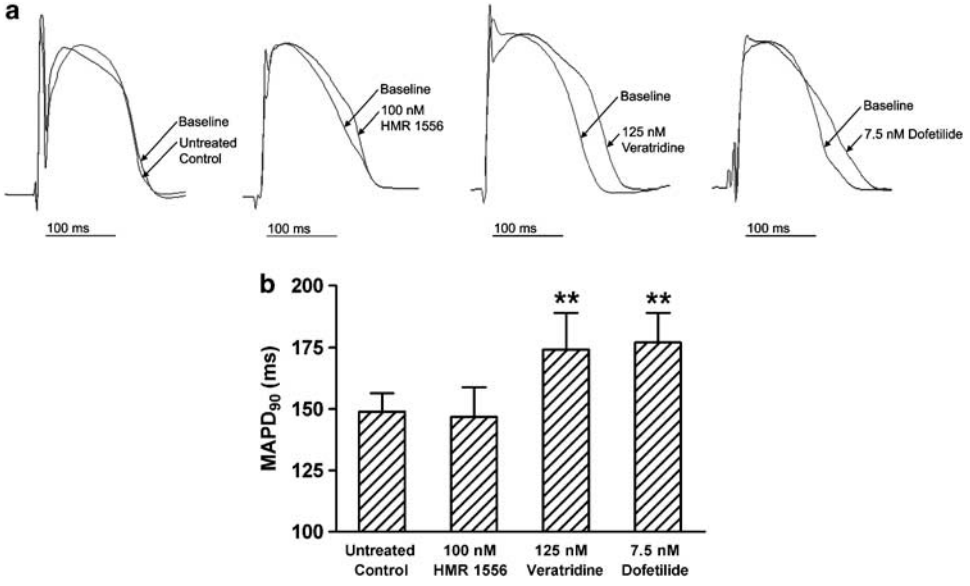

Figure 1 (**a**) Representative monophasic action potential (MAP) recordings in perfused rabbit hearts during baseline and after the individual administration of HMR 1556 (100 nM), veratridine (125 nM) or dofetilide (7.5 nM) at a pacing cycle length (CL) of 500 ms. Endocardial MAP signals were obtained from the left ventricular apex. Amplitude of the MAP recordings was rescaled for the figure. The variability in MAP morphology and amplitude can be caused by changes in contact pressure between the MAP catheter and tissue, and do not necessarily reflect changes in specific ionic currents. Such variability is consistent with the known reported feature of MAP (Franz, 1999). (**b**) Individual effects of HMR 1556, veratridine or dofetilide on MAP duration at 90% repolarization ($MAPD_{90}$) at pacing CL of 500 ms. Mean ± s.d., $n = 6$. **$P < 0.01$ vs untreated control.

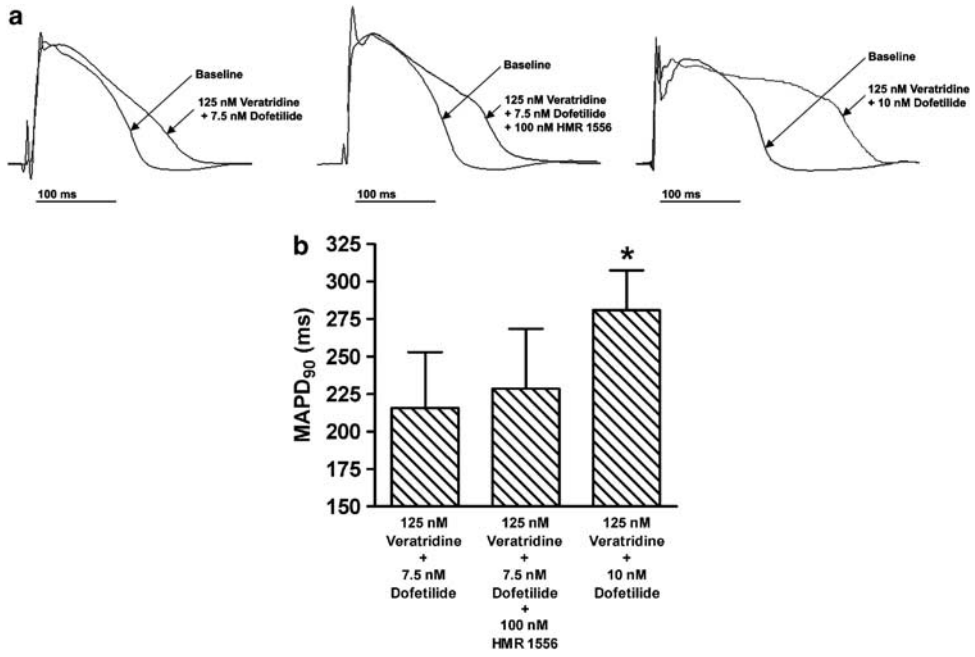

Figure 2 (**a**) Representative monophasic action potential (MAP) recordings during baseline and after the combined administration of veratridine (125 nM), dofetilide (7.5 or 10 nM) and with or without HMR 1556 (100 nM) in perfused rabbit hearts. Endocardial MAP signals were obtained from the left ventricular apex. Amplitude of the MAP recordings was rescaled for the figure. The variability in MAP morphology and amplitude can be caused by changes in contact pressure between the MAP catheter and tissue, and do not necessarily reflect changes in specific ionic currents. Such variability is consistent with the known reported feature of MAP (Franz, 1999). (**b**) Effects of combined administration of veratridine (125 nM), dofetilide (7.5 or 10 nM) and with or without HMR 1556 (100 nM) on MAP duration at 90% repolarization ($MAPD_{90}$) at pacing cycle length of 500 ms. Mean ± s.d., $n=6$. *$P<0.05$ vs 125 nM veratridine + 7.5 nM dofetilide or 125 nM veratridine + 7.5 nM dofetlide + 100 nM HMR 1556.

(7.5 nM) additively prolonged $MAPD_{90}$ ($P<0.05$, $n=6$) compared with 125 nM veratridine or 7.5 nM dofetilide alone (Figure 2). Moreover, premature beats occurred and interrupted the pacing sequence. This occasionally caused the pacing stimulus to be applied on the T wave of the preceding premature beat ('stimulus on T'), sometimes leading to pacing-induced TdP. As the incidence of TdP during pacing was dependent on the occurrence of premature beats and the number of 'stimulus on T' events, we measured the incidence of TdP in spontaneously beating hearts without pacing. The intrinsic heartbeats originate from slow ventricular escape rhythm, as the atrioventricular node had been ablated (see Methods). The spontaneous heart rate was 41 ± 13 beats per minute in the 125 nM veratridine + 7.5 nM dofetilide group and was not different from the other combined treatment groups ($P=0.19$, $n=6$).

A representative episode of spontaneous TdP and R on T premature beats after the administration of 125 nM veratridine and 7.5 nM dofetilide is illustrated in Figure 3. Although further administration of HMR to this combination increased both the number of spontaneous TdP episodes ($P<0.05$) and spontaneous R on T premature beats ($P<0.05$; Table 1), the additional $MAPD_{90}$ prolongation is not significant when compared with 125 nM veratridine + 7.5 nM dofetilide ($P=0.58$, Figure 2).

To investigate if a comparable amount of TdP could be induced by further reduction in I_{Kr} instead of blocking I_{Ks} (that is, to study the specificity of I_{Ks} in protecting against TdP development), we examined the incidence of spontaneous TdP in hearts treated with a higher concentration of dofetilide (10 nM) in the presence of 125 nM veratridine. As shown in Figure 2, despite substantial further prolongation of $MAPD_{90}$ ($P<0.05$, $n=6$) from 216 ± 37 to 281 ± 27 ms when the concentration of dofetilide was increased from 7.5 to 10 nM (added to 125 nM veratridine), no further increase in the number of TdP episodes per heart ($P=0.73$) or spontaneous R on T premature beats per heart was observed ($P=0.32$, Table 1). Despite a longer $MAPD_{90}$ ($P<0.05$) in hearts treated with veratridine + 10 nM dofetilide than in those treated with veratridine + 7.5 nM dofetilide + HMR (Figure 2), fewer spontaneous TdP episodes ($P<0.05$) were observed in the group without HMR than with HMR (Table 1). The mean number of spontaneous R on T premature beats was also less in the group without HMR than with HMR, although the difference was not statistically significant ($P=0.12$).

As spontaneous TdP and R on T premature beats may originate from a common cause (for example, early afterdepolarizations), we predicted that the greater the number of R on T premature beats, the higher would be the TdP incidence. There was a strong positive correlation ($R^2=0.92$, $P<0.0001$, $n=18$) between the number of spontaneous TdP episodes and the number of R on T premature beats (Figure 4), and that both types of proarrhythmic events were proportionately increased in hearts treated with HMR (in the presence of veratridine and dofetilide). There were no differences in the mean duration of TdP ($P=0.17$), the pause

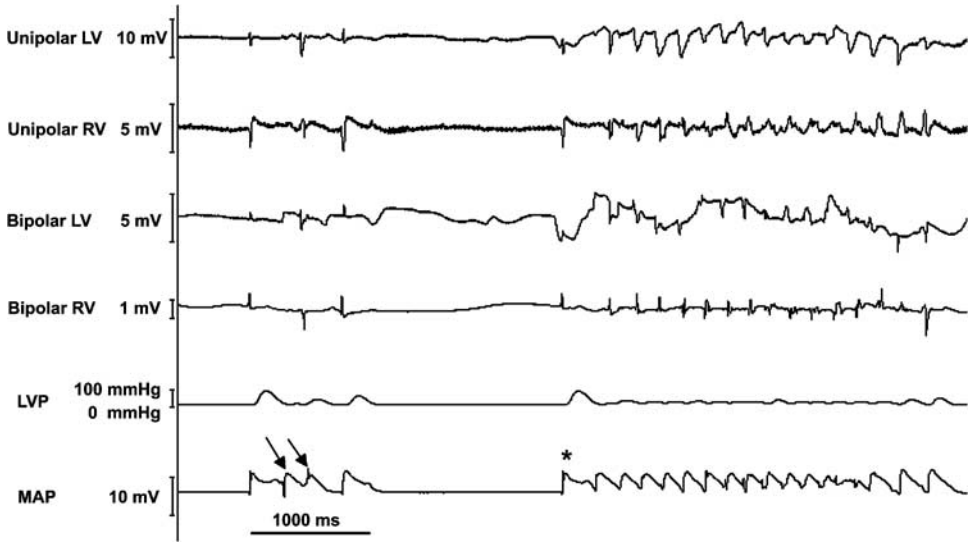

Figure 3 Representative episode of spontaneous Torsades de Pointes (TdP, indicated by *) and spontaneous R on T premature beat (indicated by arrow) in perfused rabbit hearts after the administration of 125 nM veratridine + 7.5 nM dofetilide. Unipolar and bipolar left ventricular (LV) and right ventricular (RV) electrograms were obtained from the epicardial surface, and left ventricular pressure (LVP) was obtained by inserting a fluid-filled balloon into the left ventricle. Monophasic action potential (MAP) signal was obtained from the left ventricular apex.

Table 1 Number of spontaneous Torsades de Pointes episodes and R on T premature beats in perfused rabbit ventricles

	Torsades de Pointes episodes			*R on T premature beats*		
	V+7.5D	*V+7.5D+H*	*V+10D*	*V+7.5D*	*V+7.5D+H*	*V+10D*
Mean ± s.d.	4 ± 6	22 ± 18†	5 ± 5	11 ± 16	55 ± 45‡	21 ± 16
Median (range)	2 (0–16)	20 (0–45)	3 (0–12)	3 (0–37)	56 (1–119)	25 (0–45)
No. of hearts[a]	4 in 6	5 in 6	5 in 6	4 in 6	6 in 6	5 in 6

[a]Number of hearts with at least one episode of TdP or R on T premature beats.
V = 125 nM veratridine; 7.5D = 7.5 nM dofetilide; 10D = 10 nM dofetilide; *H* = 100 nM HMR 1556.
$^{\dagger}P<0.05$ vs V + 7.5D or V + 10D; $^{\ddagger}P<0.05$ vs V + 7.5D.

duration preceding the onset of TdP ($P=0.31$) or the morphology of TdP between the three treatment groups.

As dispersion of ventricular repolarization is a predictor of TdP, we measured the baseline and post-treatment $T_{peak-end}$ interval, which is a proposed surrogate measure of dispersion of ventricular repolarization (Yan and Antzelevitch, 1998). We also measured the QT interval at pacing CL of 500 ms. Figure 5a illustrates the representative LV unipolar electrograms showing the QT and $T_{peak-end}$ interval during baseline and after drug administration. The baseline QT interval of 199 ± 19 ms was not different among the three combined treatment groups. As observed with $MAPD_{90}$, addition of 100 nM HMR to 125 nM veratridine + 7.5 nM dofetilide did not produce a significant additional QT prolongation, whereas increasing the concentration of dofetilide from 7.5 to 10 nM (in the presence of veratridine) further prolonged the QT interval ($P<0.05$, $n=6$; Figure 5b). The baseline $T_{peak-end}$ of 51 ± 10 ms was not different among the three combined treatment groups. However, the increase in $T_{peak-end}$ interval was greater ($P<0.05$, $n=6$) in the group treated with HMR compared with the other two groups without HMR (Figure 5c), indicating that adding I_{Ks} blocks significantly increased the dispersion of ventricular repolarization. There was a weak but significant correlation between the number of spontaneous TdP and the change in $T_{peak-end}$ from baseline ($R^2=0.30$, $P<0.05$, $n=18$).

As beat-to-beat instability in ventricular repolarization is another predictor of TdP (Hondeghem *et al*., 2001; Thomsen *et al*., 2006), we also assessed the stability of repolarization by measuring the s.d. of 10 consecutive measurements of $MAPD_{90}$ during pacing at a CL of 500 ms. As shown in Figure 6a, the beat-to-beat variability in $MAPD_{90}$ is greater in the veratridine + 7.5 nM dofetilide + HMR group compared with the veratridine + 7.5 nM dofetilide group (3.3 ± 3.6 vs 0.3 ± 0.3 ms, respectively, $P<0.05$, $n=6$), but not statistically significantly different from the veratridine + 10 nM dofetilide group (1.0 ± 0.8, $P=0.18$, $n=6$). The pattern of beat-to-beat variability appeared to occur in a random manner, as illustrated by Figure 6b, which shows a representative plot of individual $MAPD_{90}$ values vs the beat number in a rabbit heart treated with veratridine + 7.5 nM dofetilide + 100 nM HMR 1556.

Discussion

This study demonstrates that adding I_{Ks} block in the presence of drug-induced AP prolongation promotes TdP in

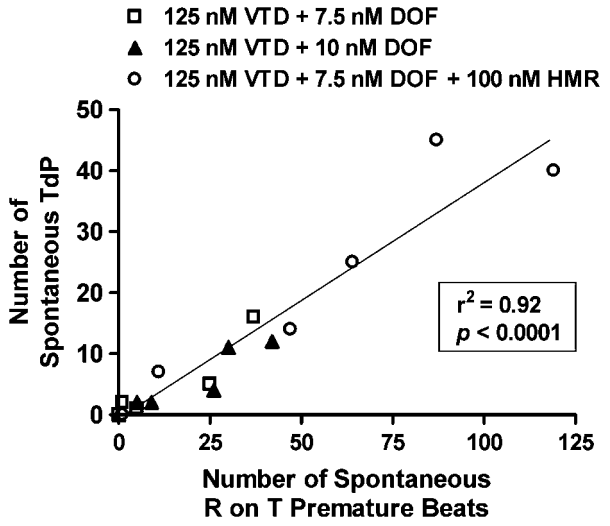

Figure 4 Correlation analysis between the number of spontaneous Torsades de Pointes (TdP) and R on T premature beats after combined administration of veratridine (VTD, 125 nM), dofetilide (DOF, 7.5 or 10 nM) and with or without HMR 1556 (HMR, 100 nM) in perfused rabbit hearts (Pearson's $R^2 = 0.92$, $P < 0.0001$). The atrioventricular nodes of the rabbit hearts were ablated to generate slow ventricular escape rhythm and the number of spontaneous TdP and R on T premature beats were measured without pacing for 10 min.

perfused rabbit hearts, but it was unlikely to be caused by additional APD prolongation. Fewer TdP were observed when APD was prolonged to a greater extent by increasing I_{Kr} block than with I_{Ks} block, indicating that there is a dissociation between the extent of drug-induced AP prolongation and incidence of TdP. Adding I_{Ks} block promotes proarrhythmia by increasing the dispersion and beat-to-beat variability in ventricular repolarization during drug-induced AP prolongation.

Reductions in I_{Ks} induced by HMR 1556 had no effect on APD when administered alone (Figure 1) and did not induce TdP in rabbit ventricles, which agrees with previous studies showing that i.v. administration of HMR 1556 alone produced little QT prolongation and did not induce TdP in anaesthetized rabbits (Lengyel *et al*., 2007; Michael *et al*., 2007; So *et al*., 2007). Drug-induced AP prolongation (for example, I_{Kr} block) prolongs the duration during which the membrane stays at a voltage range which allows I_{Ks} to activate, especially at slow heart rates (So *et al*., 2006). I_{Ks} acts as an important 'repolarization reserve' that activates and prevents excessive AP prolongation (Biliczki *et al*., 2002; Nakashima *et al*., 2004; Jost *et al*., 2005; So *et al*., 2006). However, it is unclear whether the proarrhythmic effect of I_{Ks} block during drug-induced AP prolongation is caused by additional AP prolongation or other arrhythmogenic

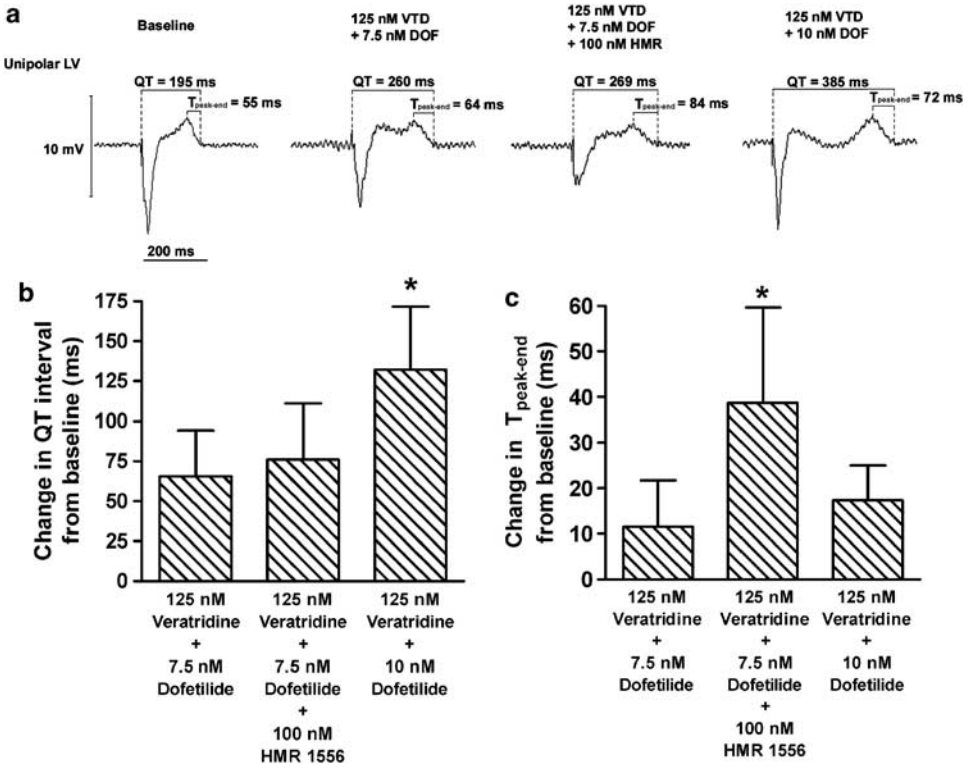

Figure 5 (**a**) Representative unipolar left ventricular (LV) electrograms during baseline and after the combined administration of veratridine (VTD, 125 nM), dofetilide (DOF, 7.5 or 10 nM) and with or without HMR 1556 (HMR, 100 nM) in perfused rabbit hearts. The QT interval and the interval between the peak and the end of T wave ($T_{peak-end}$) were measured, the later being a proposed index of dispersion of ventricular repolarization. (**b**) Summary data on the change in QT interval from baseline. (**c**) Summary data on the change in $T_{peak-end}$ from baseline. The baseline QT interval of 199 ± 19 ms and the baseline $T_{peak-end}$ of 51 ± 10 ms were not different among the three groups. QT and $T_{peak-end}$ were measured from the unipolar left ventricular electrogram during pacing at cycle length of 500 ms. Mean ± s.d., $n = 6$. *$P < 0.05$ vs veratridine + 7.5 nM dofetilide or veratridine + 10 nM dofetilide.

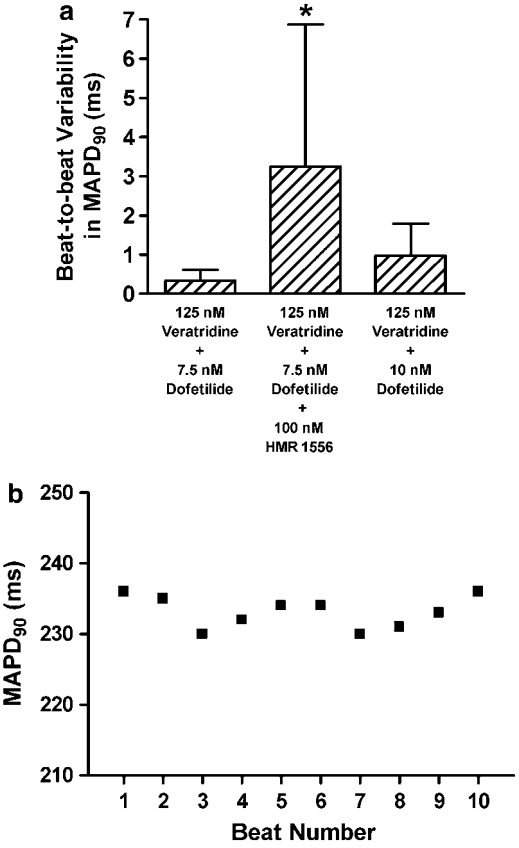

Figure 6 Effects of combined administration of veratridine (125 nM), dofetilide (7.5 or 10 nM) and with or without HMR 1556 (100 nM) on the beat-to-beat variability of ventricular repolarization during pacing at cycle length (CL) of 500 ms. (**a**) The beat-to-beat variability was measured as the s.d. of 10 consecutive monophasic action potential (MAP) duration at 90% repolarization ($MAPD_{90}$) during pacing CL of 500 ms. (**b**) A representative plot of individual $MAPD_{90}$ values vs the beat number in a rabbit heart treated with veratridine + 7.5 nM dofetilide + 100 nM HMR 1556. Mean ± s.d., $n = 6$. *$P < 0.05$ vs veratridine + 7.5 nM dofetilide.

mechanisms, such as increase in dispersion of repolarization. If adding the I_{Ks} blocker further increased APD prolongation in the presence of I_{Kr} block plus slowing of I_{Na} inactivation, one would clearly expect that the incidence of TdP would be increased. However, adding the I_{Ks} blocker did not cause further APD prolongation; thus, the incidence of TdP would not be expected to increase if additional APD prolongation is a *sine qua non* for increase in TdP occurrence. Therefore, the ability of I_{Ks} to prevent TdP development may not be solely related to its ability to limit drug-induced AP prolongation, as a longer AP produced by increasing I_{Kr} block (from 7.5 to 10 nM dofetilide in the presence of veratridine) produces less TdP compared with the hearts treated with the I_{Ks} blocker (Figure 2 and Table 1). There is, thus, a dissociation between the extent of drug-induced AP prolongation and TdP incidence, and other arrhythmogenic mechanisms may be responsible for the proarrhythmic effect of I_{Ks} block. In a recent study by Lengyel *et al*. (2007), adding I_{Ks} block to I_{Kr} block produced an additional corrected QT prolongation by 8% in rabbits *in vivo*, and the combined I_{Kr} and I_{Ks} block elevated the incidence of TdP (82%) compared with either I_{Kr} block (28%) or I_{Ks} block alone (0%). The threefold increase in TdP (from 28 to 82%) is unlikely to be caused by the relatively small increase in corrected QT interval alone, suggesting that other proarrhythmic mechanisms of I_{Ks} blockade may be responsible. By contrast, under our experimental conditions, which involve previous blockade of I_{Kr} and slowing of I_{Na} inactivation, I_{Ks} block did not produce a significant additional AP prolongation. However, adding I_{Ks} block markedly increased the number of TdP episodes, from a median of 2 to 20 episodes per heart (Table 1). Our data suggest that I_{Ks} block promotes TdP out of proportion to its AP prolonging effect, and that there is not a simple linear relationship between the incidence of TdP and the absolute amount of AP prolongation. Hondeghem (2007) has proposed that QT prolongation itself is antiarrhythmic, but it is usually associated with other proarrhythmic indices, such as triangulation of the AP, reverse use dependence, AP instability and dispersion of repolarization (known as TRIaD); QT prolongation appears to account for only some of the phenomena associated with TdP.

In the combined drug studies, we increased the concentration of dofetilide from 7.5 to 10 nM in an attempt to produce a small increment in APD. However, we observed that $MAPD_{90}$ prolonged by ~30%, indicating that the concentration range was likely at the very steep portion of the concentration–response curve. The large magnitude of response may also be owing to the synergistic effects of veratridine and dofetilide. Veratridine + 10 nM dofetilide was able to produce a much longer $MAPD_{90}$, but nevertheless induced less TdP compared with the HMR 1556-treated group (Figures 2 and Table 1).

Why would adding I_{Ks} block to veratridine and 7.5 nM dofetilide highly promote the TdP incidence without causing significant APD prolongation, whereas increasing I_{Kr} block from 7.5 to 10 nM dofetilide (in the presence of veratridine) substantially further prolonged APD without increasing the incidence of TdP? One mechanism may be that I_{Ks} block, but not additional I_{Kr} block, increases dispersion of ventricular repolarization. This is supported by our observation that adding an I_{Ks} blocker to veratridine and 7.5 nM dofetilide increased the $T_{peak-end}$ interval to a greater extent than increasing I_{Kr} block from 7.5 to 10 nM dofetilide (in the presence of veratridine) (Figure 5). This also agrees with an *in vitro* study that directly measured transmural dispersion in a canine ventricular wedge model, where combined I_{Kr} and I_{Ks} blockade increased transmural dispersion of ventricular repolarization compared with I_{Kr} block alone (Burashnikov and Antzelevitch, 2002). The mechanism for the apparent increase in transmural dispersion of repolarization by adding the I_{Ks} blocker is unclear. I_{Ks} is heterogeneously expressed with less I_{Ks} in the mid-myocardium than the epicardium and endocardium (Liu and Antzelevitch, 1995). On the basis of this observation, one would predict that I_{Ks} block may induce less APD prolongation in the mid-myocardium (and thus less dispersion of repolarization). However, Shimizu and Antzelevitch (1998) showed that administering the selective I_{Ks} blocker chromanol 293B alone prolonged APD homogeneously across the three myocardial layers and therefore did not affect the transmural dispersion. The same research group (Liu and Antzelevitch, 1995) suggested that the lowest

I_{Ks} expression in the mid-myocardium may cause the APD to be the longest, but a long APD may allow more functional I_{Ks} activation during positive membrane potentials. Moreover, the effect of adding an I_{Ks} blocker to I_{Kr} block on dispersion may be different from that of I_{Ks} block alone. I_{Kr} blockers have been shown to exert the greatest APD prolongation in the mid-myocardium, particularly during slow heart rates (Shimizu and Antzelevitch, 1997). Therefore, the combined I_{Kr} block and slowing of I_{Na} inactivation is likely to induce greater APD prolongation in the mid-myocardium, and hence allow more time for I_{Ks} to activate in this myocardial layer. I_{Ks} is therefore expected to be most functionally activated in the mid-myocardium and has a critical function as the 'safety current', thereby minimizing transmural dispersion of repolarization during bradycardia.

Another potential contributing factor to the increased TdP incidence is the increase in beat-to-beat variability of APD observed following I_{Ks} block, as variability in ventricular repolarization is another known predictor of TdP (Hondeghem *et al*., 2001; Thomsen *et al*., 2006). Our results are consistent with the recent study by Lengyel *et al*. (2007), where adding I_{Ks} block to I_{Kr} block greatly increased the beat-to-beat variability of the QT interval in anaesthetized rabbits. We observed that the pattern of beat-to-beat variation is random rather than occurring regularly on alternating beats, which agrees with previous studies (Thomsen *et al*., 2006; Lengyel *et al*., 2007). The magnitude of the beat-to-beat variability of APD is small, probably because it was measured at a period with electrical pacing at a constant CL, without interruptions by premature beats or spontaneous changes in heart rate as observed *in vivo*. The mechanism underlying the instability of ventricular repolarization with I_{Ks} block may be related to an increase in the slope of ventricular effective refractory period vs CL relationship (reverse rate-dependence) after combined $I_{Kr} + I_{Ks}$ block compared with I_{Kr} block alone (So *et al*., 2006). Increase in the steepness of the reverse rate-dependence enhances the oscillations in ventricular repolarization, which may be associated with proarrhythmias (Hondeghem *et al*., 2001).

Our studies are based on the experimental approach of drug perfusion in isolated rabbit ventricles using the measurement of MAP without direct measurement of the transmembrane AP or ionic currents. Unlike the transmembrane AP, variation in the MAP can be caused by changes in contact pressure between the MAP catheter and the tissue, and do not necessarily reflect changes in specific ionic currents (Franz, 1999). Thus, the use of MAP measurement is limited to estimating the overall duration of ventricular repolarization, and the morphology of MAP provides little direct evidence regarding ionic mechanisms compared with transmembrane AP measurements. There are well-known species differences in the expression and kinetics of I_{Ks}; although there are several important differences between the human and rabbit hearts (for example the transient outward K^+ current (I_{to}) in rabbits has a slower recovery from inactivation than in humans (Lu *et al*., 2001)), rabbit hearts are similar to human hearts in terms of the activation and deactivation kinetics of I_{Ks} (Jost *et al*., 2007). Moreover, episodes of TdP may inhibit the onset of subsequent TdP because the QT is transiently shortened. As there were more TdP episodes in the group treated with HMR 1556 (plus I_{Kr} block and slowing of I_{Na} inactivation), such a mechanism would lead to an underestimation of the number of TdP episodes in this experimental group.

Our data suggest that I_{Ks} preservation is crucial in protecting against TdP development. This result agrees with the suggestion by Salata *et al*. (1998) that I_{Ks} activation may prevent the development of proarrhythmia by limiting excessive APD prolongation caused by other K^+ channel blockers. During the rapid phase 3 repolarization, I_{Ks} channels deactivate slowly (Sanguinetti and Jurkiewicz, 1990; Lengyel *et al*., 2001), leading to a residual I_{Ks} conductance after the onset of ventricular repolarization, which decreases membrane excitability (Davidenko *et al*., 1994; Beaumont *et al*., 1995). This may counterbalance the depolarizing calcium current, which can reactivate during the late phase of repolarization to trigger early afterdepolarizations. A similar mechanism has been proposed for preventing triggered arrhythmias by the inward rectifier K^+ current (Pogwizd *et al*., 2001). This mechanism is also consistent with previous studies showing that adding I_{Ks} blockers to an already prolonged APD promotes early afterdepolarizations (Burashnikov and Antzelevitch, 2002), whereas adding an I_{Ks} activator (L3) decreases the incidence of early afterdepolarizations in dofetilide-treated and hypertrophied ventricular myocytes (Xu *et al*., 2002). I_{Ks} block may also change the AP morphology such that the membrane potential resides longer at the voltage range that may promote activation of depolarizing currents and trigger TdP. Future studies using direct transmembrane AP measurements or computer modelling are needed to assess the change in AP morphology in these settings. Early afterdepolarizations and TdP generation may thus reflect an imbalance between inward and outward currents during phase 3 repolarization, rather than being purely a consequence of APD prolongation (Antzelevitch, 2004; Hondeghem, 2006). Sympathetic activation has also been suggested to induce early afterdepolarizations by reactivation of L-type calcium current, which may act as a trigger for TdP (January and Riddle, 1989). It may also increase the dispersion of repolarization owing to heterogeneous distribution of both sympathetic innervation and I_{Ks} channels in human myocardium (Kawano *et al*., 2003; Szentadrassy *et al*., 2005). On the other hand, β-adrenoceptor stimulation increases I_{Ks} magnitude and accelerates its activation kinetics (Volders *et al*., 2003), which may be a protective mechanism against TdP induction during drug-induced APD prolongation. Sympathetic denervation in perfused hearts or other *in vitro* preparations limits the ability to assess the role of β-adrenoceptor stimulation in L-type calcium current and I_{Ks} activation, and their overall effects on TdP induction. Therefore, future *in vivo* studies are necessary to investigate the role of sympathetic activation in the torsadogenic mechanism of I_{Ks} block.

In conclusion, this study highlights the function of I_{Ks} in protecting against TdP development under the condition of substantial APD prolongation. I_{Ks} acts as a 'safety current', or repolarization reserve, which may prevent the development of proarrhythmic events when APD is prolonged. Because I_{Ks} contributes little to ventricular repolarization during

baseline state ('latent nature') (Biliczki *et al*., 2002; Jost *et al*., 2005; So *et al*., 2006), some individuals with I_{Ks} channel mutations may have normal baseline QT interval but a higher risk of TdP development upon exposure to drugs that inhibit repolarizing currents, especially I_{Kr} (Roden, 2006). The use of I_{Ks} blockers could promote the occurrence of TdP, especially when other repolarizing currents are reduced by drugs, genetic mutations or heart failure.

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Conflict of interest

The authors state no conflict of interest.

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