Effects of Ranolazine on Torsades de Pointes Tachycardias in a Healthy Isolated Rabbit Heart Model

Samuel Sossalla, Nora Wallisch, Karl Toischer, Christian Sohns, Dirk Vollmann, Joachim Seegers, Lars Lüthje, Lars S. Maier & Markus Zabel

1 Klinik für Kardiologie und Pneumologie/Herzzentrum, Georg-August-Universität Göttingen, Göttingen, Germany
2 DZHK (German Center for Cardiovascular Research), Göttingen, Germany
3 Innere Medizin II – Kardiologie, Universitätsklinikum Regensburg, Regensburg, Germany

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Correspondence
Prof. Dr. med. Markus Zabel, Department of Cardiology and Pneumology/Heart Center Göttingen, Georg-August-University Göttingen, Robert-Koch-Street 40, 37075 Göttingen, Germany.
Tel.: +49/551/39-10265
Fax: +49/551/39-19127
E-mail: markus.zabel@med.uni-goettingen.de

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ABSTRACT

Purpose: Torsades de pointes (TdP) tachycardias are triggered, polymorphic ventricular arrhythmias arising from early afterdepolarizations (EADs) and increased dispersion of repolarization. Ranolazine is a new agent which reduces pathologically elevated late I_{Na} but also I_{Kr}. Aim of this study was to evaluate the effects of ranolazine in a validated isolated Langendorff-perfused rabbit heart model. Methods: TdP was reproducibly induced with d-sotalol (10^{-4} mol/L) and low potassium (K) (1.0 mmol/L for 5 min, pacing at CL 1000 ms). In 10 hearts, ECG and 8 epi- and endocardial monophasic action potentials were recorded. Action potential duration (APD) was measured at 90% repolarization and dispersion defined as APD max–min. Results: D-sotalol prolonged APD_{90} and increased dispersion of APD_{90}, simultaneously causing EADs and induction of TdP. The combination of d-sotalol and two concentrations of ranolazine did not increase dispersion of ventricular APD_{90} as compared to vehicle. Ranolazine at 5 μmol/L did not cause additional induction of EADs and/or TdP but also did not significantly suppress arrhythmic triggers. The higher concentration of ranolazine (10 μmol/L) in combination with d-sotalol caused further prolongation of APD_{90}, at the same time reduction in APD_{90} dispersion. In parallel, the incidence of EADs was reduced and an antitorsadogenic effect was seen. Conclusions: In the healthy isolated rabbit heart (where late I_{Na} is not elevated), ranolazine does not cause proarrhythmia but exerts antiarrhythmic effects in a dose-dependent manner against d-sotalol/low K-induced TdP. This finding—despite additional APD prolongation—supports the safety of a combined use of both drugs and merits clinical investigation.

Introduction

Drug-induced Torsades de Pointes (TdP) can be caused by multiple compounds and has become an important issue in current pharmacological development. Manifestation of TdP is a classic proarrhythmic effect of class III antiarrhythmic drugs such as sotalol. Although occurring rarely, TdP represents a potentially lethal polymorphic ventricular tachycardia.

TdP usually manifest in conjunction with QT-interval prolongation or if patients present with particular risk factors. Many antiarrhythmic drugs have the ability to prolong action potential (AP) duration (APD) and refractoriness by inhibition of K-currents when most needed, at fast heart rates, thereby compromising their antiarrhythmic effects. Reverse use-dependence of APD is a physiological capacity of the healthy heart wherein the APD usually shortens at increasing heart rate. Some antiarrhythmic drugs also exhibit a reverse use-dependent effect meaning the ability of the drug to preferentially prolong cardiac APD rather at slow than at fast heart rates [1,2].

Under normal conditions, most Na channels open only transiently and become quickly inactivated, thereby producing the peak Na current which produces the upstroke of the AP. There is increasing recognition of the importance of this small late component of I_{Na} (late I_{Na}) due to Na channels that remain active, inactivate with much slower kinetics, or reopen. The amplitude of this current is small in healthy myocardium but may persist for hundreds of milliseconds (i.e., during the whole AP) [3]. Therefore, the long persistence and its slow inactivation kinetics make late I_{Na} relevant [4,5] when increased as it has been shown in pathological conditions such as ischemia, hypoxia, elevated reactive oxygen, heart failure, and nitrogen species [6–10].

Late I_{Na} has been shown to act as a potent contributor to severe arrhythmias and its pharmacological inhibition seems to be of antiarrhythmic potential [11] (for review see [3]). An elevated late I_{Na} causes prolongation of the cardiac AP making early afterdepolarizations (EADs) more likely to occur [12,13]. Additionally, cellular Na cycling is tightly integrated with Ca homeostasis, as Na modulates the transport direction of the Na/Ca exchanger (NCX).
in addition to the membrane potential. Cellular Na overload and AP prolongation stimulate the reverse mode NCX facilitating the efflux of Na in exchange for influx of Ca [14]. The net result is an elevated diastolic Ca concentration making late afterdepolarizations (DADs) more likely to occur via “leaky” cardiac ryanodine receptors (RyR2) of the sarcoplasmatic reticulum and NCX which possibly generates a depolarizing current (transient inward current, \( I_{\text{Na}} \)) that give rise to delayed afterdepolarizations (DADs) [6,13–16].

Ranolazine is currently the most potent clinical inhibitor of late \( I_{\text{Na}} \) [3]. In multicellular tissue and in cardiac myocytes, ranolazine was shown to cause direct and indirect concentration-, voltage-, as well as frequency-dependent inhibition of late \( I_{\text{Na}} \) [17–20]. In cardiomyocytes isolated from failing canine hearts, ranolazine was found to be 38-fold more potent in inhibiting late than peak \( I_{\text{Na}} \); 50% inhibition of peak and late \( I_{\text{Na}} \) occur at concentrations of 244 and 6.5 \( \mu \)mol/L, respectively [17]. However, it remains unclear how this drug produces its selective effect on late \( I_{\text{Na}} \).

It is important to note that ranolazine also has inhibitory effects on \( I_{\text{K}} \), which would rather prolong cardiac APD [20,21]. This is very likely the basis for a moderate QT-interval prolongation in patients treated with ranolazine [22]. Because of concerns regarding QT-interval prolongations, ranolazine is currently contraindicated in combination with class III antiarrhythmic drugs apart of amiodarone. On the basis of the particular pharmacological action that this drug has, the present study was designed to investigate the net effect of ranolazine on APD and ventricular APD dispersion in healthy isolated rabbit hearts where late \( I_{\text{Na}} \) is not relevantly elevated [18]. Furthermore, another objective of this study was to investigate the dose-dependent safety of ranolazine in combination with sotalol, both of which are known to inhibit \( I_{\text{K}} \)-channels, under physiological conditions and in the presence of low K in order to test for TdP formation.

**Methods**

**Animals and Materials**

New Zealand white rabbits (3.5–5.0 kg) were used. All procedures regarding care and use of animals were in accordance with institutional guidelines. Rabbits were heparinized and anaesthetized with sodium thiopental (50 mg/kg/i.v). Hearts were rapidly cannulated and perfused with oxygenized Tyrode’s solution containing 1.2 mmol/L and stimulation to a CL of 1000 ms. When triggered arrhythmias ensued, pacing was discontinued so as not to disturb these. After a washout period, the same protocol was repeated as before but now with a solution containing either 1.2 mmol/L d-sotalol and finally d-sotalol and ranolazine were exposed to the same heart after washout periods. After achieving steady state at CL of 1000 ms, the pacing CL was decreased step-wise to 800, 500, and 400 ms. Steady state at a certain CL was considered after 100 beats, which had to be undisturbed by premature ventricular contractions for at least 25 beats. After completion of the baseline recordings in 20 experiments, d-sotalol was added to the buffer solution: d-sotalol was applied at a concentration of 10\(^{-4}\) mol/L, and the stimulation protocol was repeated again. Then, the solution was switched to a low K solution containing 1.2 mmol/L and stimulation to a CL of 1000 ms. When triggered arrhythmias ensued, pacing was discontinued so as not to disturb these. After a washout period, the same protocol was repeated as before but now with a solution containing either ranolazine at 5 or 10 \( \mu \)mol/L ion addition to d-sotalol.

**Statistics**

All data are shown as mean ± SEM. Student’s t-test or two-way repeated-measure ANOVA with post hoc tests was used to test for significance. A two-sided \( P \) value of <0.05 was considered significant.

**Results**

**Effects on Whole Heart Action Potential Duration**

Isolated rabbit hearts were superfused and paced with decreasing cycle lengths. Action potential duration measured at 90% repolarization (APD\(_{90}\)) was prolonged from 205 ± 24 ms to 238 ± 22 ms (CL 800 ms) in the presence of 10\(^{-3}\) mol/L d-sotalol.
Effects on Whole Heart Action Potential Dispersion

Dispersion of APD\textsubscript{90} was increased after temporally lowering K concentration (1.0 mmol/L for 5 min) during bradycardic pacing at CL 1000 ms. D-sotalol alone and in combination with either 5 or 10 µmol/L ranolazine largely increased APD\textsubscript{90} dispersion under low K concentrations (Figures 3A,C, \( P < 0.05 \) vs. baseline, \( n = 5 \) each). Before TdP was induced, steady-state pacing was carried out at CLs of 400, 500, 800, and 1000 ms. Figure 3B depicts that d-sotalol markedly increased dispersion of APD\textsubscript{90} at a CL of 800 ms from 37 ± 4 ms to 72 ± 10 ms (\( P < 0.01 \)). Addition of 5 µmol/L ranolazine to d-sotalol reversed this effect to 48 ± 8 ms which was not statistical different to vehicle (Figure 3B, \( P = 0.26 \) RM-ANOVA). Conversely, addition of 10 µmol/L ranolazine to d-sotalol reduced APD\textsubscript{90} dispersion compared with d-sotalol alone (Figure 3D, \( P < 0.05 \) RM-ANOVA).
Effects on EAD and TdP Inducibility

No EADs were seen under steady-state conditions with normal potassium concentration. Upon lowering K to 25% for 5 min, EADs and TdP could be observed in all experiments. EADs were counted for 120s in each experiment. Interestingly, there was no difference in the amount of EADs in the presence of d-sotalol alone or in combination with 5 μmol/L ranolazine. An average of 108 ± 5 EADs/120s occurred in the presence of d-sotalol compared with 104 ± 14/120s when 5 μmol/L ranolazine was added to the solution (Figure 4E, *P = 0.79). However, the higher concentration of ranolazine was capable to largely reduce the occurrence of EADs triggered by low K solution and d-sotalol. In these experiments, addition of 10 μmol/L ranolazine largely reduced the occurrence of EADs from 99 ± 13/120 s to 45 ± 12/120 s (Figure 4G, *P < 0.05).

TdP was defined as runs of polymorphic VT that consist of at least three beats. Under normal conditions without low K solution, no TdP was observed. Upon induction, TdP coincided with increased EADs, APD, and dispersion. As Figure 4F presents, hearts that were treated with d-sotalol alone developed 9 ± 3 TdP/experiment. Addition of 5 μmol/L ranolazine caused a trend toward a reduction in the incidence of TdP to 5 ± 3/ experiment that was, however, not statistically significant (*P = 0.37). In another series of experiments, we doubled the concentration of ranolazine to 10 μmol/L. Interestingly, now ranolazine effectively suppressed the incidence of TdP from 4 ± 1/experiment to 0.6 ± 0.6/experiment (Figure 4H, *P < 0.05).

Discussion

The present study was designed to investigate the electrophysiological consequences and safety of a combination of the class III antiarrhythmic drug d-sotalol and ranolazine in a validated isolated Langendorff-perfused rabbit heart model with the following major findings: (1) d-sotalol prolonged APD and significantly increased the APD dispersion thereby causing induction of EADs and TdP. (2) The combination of d-sotalol with 5 μmol/L ranolazine did not further prolong APD and lacked the induction of EADs and/or TdP but, however, did not significantly suppress arrhythmogenic triggers. (3) The higher concentration of ranolazine (10 μmol/L) in combination with d-sotalol caused further prolongation of the APD, but significantly reduced APD dispersion and most importantly the incidence of EADs and triggered arrhythmias and consequently exhibited antitorsadogenic effects.

The Isolated Langendorff-Perfused Rabbit Heart Model

Our isolated Langendorff-perfused rabbit heart model provides a simple crystalloid-perfused isolated heart that allows for simultaneous recordings of multiple MAP signals from the myocardium (both endocardium and epicardium of both ventricles) [25]. As a whole heart model, it is therefore well suited to observe the generation of arrhythmias from local early afterdepolarizations (EADs) and in the circumstances of an increased dispersion of repolarization such as torsade de pointes (TdP) arrhythmias usually are [26,27]. Verification of the occurrence of TdP arrhythmias can then be performed using the simultaneous multilead volume-con-
Figure 4 Effects of sotalol and ranolazine on formation of EADs and TdPs. (A) Representative original tracing showing EADs in a d-sotalol-treated heart. (B–D) Original recordings showing that TdP occurred in d-sotalol-treated hearts, to a lower extend when 5 µmol/L ranolazine was added and could be suppressed in the presence of 10 µmol/L ranolazine. (E) Mean values of EADs in hearts treated with sotalol alone or in combination with 5 µmol/L ranolazine. (F) Mean values of TdP incidence in hearts treated with sotalol alone or in combination with 5 µmol/L ranolazine. (G) Mean values of EADs in hearts treated with sotalol alone or in combination with 10 µmol/L ranolazine (§P < 0.05). (H) Mean values of TdP incidence in hearts treated with sotalol alone or in combination with 10 µmol/L ranolazine (§P < 0.05).
ducted ECG of the model. This has been used to correlate even simple ECG indexes, such as an increased dispersion of the QT interval and an increased T wave area, to the occurrence of triggered arrhythmias [23,24]. The same ECG indexes may also be measured in patients and may serve as measures of proarrhythmic risk in patients treated with drugs that prolong action potential duration.

Differential Modulation of the APD

D-sotalol is a class III antiarrhythmic potassium channel blocker affecting the rapidly activating delayed rectifier K current (IKr) but has also beta-blocking capacities. D-sotalol is commonly used for rhythm-control treatment of atrial fibrillation (AF). The combination of class III antiarrhythmic drugs apart from amiodarone with ranolazine is currently contraindicated making our findings of clinical relevance in particular due to the fact that recent studies suggest that ranolazine may also be of therapeutically interest for the treatment of AF [3,28–32].

D-sotalol caused a significant prolongation of the APD in our model. Addition of 5 µmol/L ranolazine did not pronounce this effect while 10 µmol/L caused a further prolongation. This finding of a dose-dependent effect of ranolazine on the cardiac APD is remarkable. Although ranolazine also has at least weak peak IKr inhibiting effects, this compound has an up to 38-fold higher potency for late IKr than peak IKr with an IC50 of 6.5 vs. 244 µmol/L [17] in ventricular myocytes with a therapeutic range of ranolazine of 2–8 µmol/L. Inhibition effects of ranolazine on late IKr was demonstrated both indirectly and directly in multicellular myocardium and in isolated myocytes in several diseased conditions [17–19]. This capacity would lead to an abbreviation of the APD due to inhibition of a net inward current which has not been observed in our study. Similarly to d-sotalol, ranolazine also inhibits IKs in cardiomyocytes with a potency of ~12 µmol/L [20,21]. Blocking IKs causes prolongation of the ventricular APD, whereas inhibition of late IKs has the opposite effect and abbreviates the AP. Therefore, the net effect of ranolazine on APD is mainly driven by the relative magnitude of the reductions in late IKs (inward) and IKr (outward) currents during the repolarization period [3]. Of note, it has been also shown that ranolazine exerts differential effects on APD throughout the ventricular wall due to differences in late IKs amplitude [20]. Because we used healthy rabbit hearts where late IKs amplitude is small [18], ranolazine caused no APD abbreviation in contrast to models with elevated late IKs such as heart failure, ischemia and when late IKs is pharmacologically elevated [10,17–19,33]. It is, however, interesting to note that potent blockade of IKs by ranolazine outweighed its effect on late IKs leading to a further net APD prolongation when d-sotalol was combined with 10 µmol/L ranolazine. Our results are in line with the findings of a recent study investigating the effects of ranolazine in addition to different class III antiarrhythmic drugs. Frommeyer and coworkers also report that 10 µmol/L ranolazine further increased the APD compared with d-sotalol alone without investigating lower concentrations of ranolazine [34]. The dose dependency revealed in our study might be attributed to the different IC50 values of ranolazine for IKr and late IKs. On the one hand, 5 µmol/L ranolazine is closed to the reported IC50 of 6.5 µmol/L to inhibit late IKs making a potent inhibition very likely, while the IC50 to inhibit IKr is about 12 µmol/L [17,20,21]. On the other hand, 10 µmol/L ranolazine is closer to the IC50 to inhibit IKr making a more potent inhibition of this current and consequent APD prolongation very likely.

Antitorsadogenic Effects of Ranolazine

Drug-induced TdP has emerged as one of the most significant concerns in the safety of compounds and a major obstacle to new drug development. Occurrence of TdP is the classic proarrhythmic effect of class III drugs as d-sotalol that has been causally linked to an increased dispersion of ventricular repolarization [35]. D-sotalol was shown to cause a rate-dependent and concentration-dependent increase in dispersion of ventricular repolarization in our isolated rabbit heart model [23,24]. Both drugs, d-sotalol and ranolazine, are capable to inhibit IKr, thereby prolonging the QT interval of the ECG in patients (modestly with ranolazine) [22]. This is the reason why it is important to determine whether the combination causes a more pronounced and thus probably dangerous dispersion of ventricular APD than d-sotalol alone. Here, we show that the increased dispersion of ventricular APD well investigated for d-sotalol does not become potentiated in combination with ranolazine. The contrary is the case: ranolazine was capable to reduce the increased dispersion of ventricular APD as it was also reported by Frommeyer and coworkers earlier [34]. Another study shows that endogenous (physiological) late IKr although small in amplitude, indeed modulates ventricular repolarization, APD, and arrhythmogenesis when repolarization reserve was decreased by treatments of rabbit hearts with E-4031, 4-aminoypyridine, and BaCl2 to decrease Ito, IKr, and IK1, respectively [36]. In their experiments, tetrodotoxin or ranolazine significantly decreased APD, transmural dispersion of repolarization, beat-to-beat variability, and the QT interval. Taken together with our findings, it can be stated that endogenous late IKs seems to be arrhythmogenic in hearts with decreased repolarization reserve and that inhibition of this small current using ranolazine reduces arrhythmogenic triggers. Accordingly, we found a decreased occurrence of EADs when 10 µmol/L ranolazine was added to d-sotalol.

Episodes of TdP was induced by means of bradycardia, a high concentration of d-sotalol, and transient reduction in K concentrations in the perfusate. The induction was reproducible when the protocol was repeated. All of the inducing factors as well as the intermediate CL and particularly the polymorphic undulating pattern of the simultaneous ECG suggest that the arrhythmias induced are similar to TdP observed in the clinical setting [23,24]. Transmural differences of late IKs and hence APD might increase transmural dispersion of repolarization and QT interval, which underlies the development of TdP tachyarrhythmias [37]. The potential of ranolazine to suppress EADs induced by IKr blockers which are considered as triggers for the initiation of proarrhythmia has been reported in a previous study [19]. It may be attributed to the inhibition of the physiologically late IKs, which becomes a more pronounced contributor of ventricular repolarization when IKr and/or IKs are reduced [36]. Probably different amplitudes of late IKs throughout the LV wall may also account for our observations. Late IKs has been supposed to be largest in

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midmyocardial myocytes that can be characterized by having APs that prolong disproportionately relative compared with the APs of other LV myocytes in response to drugs that prolong QT interval [38]. Accordingly, ranolazine produced a preferential abbreviation of midmyocardial APD, thereby causing a reduction in transmural dispersion of repolarization [20]. Finally, our findings confirm previous reports showing a potent suppression of TdP in vitro in isolated rabbit hearts pretreated with d-sotalol, in vivo in anesthetized rabbits treated with the I_{Kr} blocker clofilium and in a chronic atioventricular block dog model where TdP was induced using doxetilide [34,39,40]. The latter one also found a dose dependency of ranolazine to suppress arrhythmogenic triggers.

Clinical Implications

Ranolazine has been reported to cause a slight prolongation of the ECG QT interval in patients [22]. In general, extended QT intervals increase the risk of sudden cardiac death. Because of this, ranolazine is contraindicated in combination with class III antiarrhythmic drugs apart of amiodarone. Our findings challenge this contraindication due to the fact that addition of ranolazine in both concentrations did neither potentiate arrhythmogenic triggers nor increase the likelihood of TdP. The contrary is the case, 10 μmol/L ranolazine in addition to d-sotalol sufficiently suppressed the occurrence of TdP in our model although further APD prolongation was present. This finding further substantiates that ranolazine is a safe drug despite of its I_{Kr}-blocking properties at least in our rabbit model. Combination of ranolazine with amiodarone has been reported in studies to be safe [31] and is approved. To our knowledge, there is no study in men which investigates the safety and efficacy of a combination with d-sotalol and ranolazine for the treatment of arrhythmias. Only one clinical trials was recently performed (HARMONY, EudraCT-Nr: 2011-001134-42), investigating ranolazine alone or in combination with dronedarone with respect to atrial fibrillation burden in subjects with paroxysmal atrial fibrillation and implanted pacemakers. Another trial RAFFAELLO (EudraCT-Nr: 2011-002789-18) is a dose-ranging study testing the efficacy and safety of three doses of ranolazine versus placebo in maintaining sinus rhythm after successful electrical cardioversion in subjects with persistent atrial fibrillation.

Application of ranolazine does not cause an increase in cardiovascular death or all-cause mortality in the Metabolic Efficiency with Ranolazine for Less Ischemia in Non-ST-Elevation Acute Coronary Syndromes–Thrombolysis In Myocardial Infarction 36 (MERLIN–TIMI 36) trial. Antiarrhythmic effects of ranolazine were observed in this study by using continuous Holter monitoring. There was a ~36% reduction in ventricular tachycardia lasting ≥8 beats in ranolazine-treated patients [11]. Most importantly for our study, the incidence of sudden cardiac death was significantly elevated in the placebo group with a QTc of more than 450 ms, but not in patients taking ranolazine. Moreover, in patients with impaired ejection fraction (EF < 40%), sudden cardiac death was markedly but not significantly (P = 0.07) reduced by ~45% in patients treated with ranolazine.

Taken together, ranolazine appears to have antiarrhythmic ventricular effects in patients after admission for ACS. Nevertheless, studies specifically designed to evaluate the potential role of ranolazine as an antiarrhythmic agent on ventricular arrhythmias and in combination with other antiarrhythmic drugs are warranted.

Conclusion

Our results in the healthy rabbit in vitro model clearly show that ranolazine in addition to d-sotalol does not potentiate the proarrhythmic risk of d-sotalol related to I_{Kr} blockade although APD is further prolonged by the combination with 10 μmol/L ranolazine. This is likely explained by its inhibitory effects on late I_{Na} of ranolazine. Clinical studies are clearly needed to investigate the role and safety of this drug on top of class III antiarrhythmic agents in order to prevent proarrhythmia but also as a new treatment option of ventricular and atrial arrhythmias.

Conflict of Interest

Dres. Sossalla and Maier receive speaker’s honoraria from Berlin-Chemie.

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