Original Article

Effects of Late Sodium Current Blockade on Ventricular Refibrillation in a Rabbit Model

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Background—After defibrillation of initial ventricular fibrillation (VF), it is crucial to prevent refibrillation to ensure successful resuscitation outcomes. Inability of the late Na+ current to inactivate leads to intracellular Ca2+ dysregulation and arrhythmias. Our aim was to determine the effects of ranolazine and GS-967, inhibitors of the late Na+ current, on ventricular refibrillation.

Methods and Results—Long-duration VF was induced electrically in Langendorff-perfused rabbit hearts (n=22) and terminated with a defibrillator after 6 minutes. Fibrillating hearts were randomized into 3 groups: treatment with ranolazine, GS-967, or nontreated controls. In the treated groups, hearts were perfused with ranolazine or GS-967 at 2 minutes of VF. In control experiments, perfusion solution was supplemented with isotonic saline in lieu of a drug. Inducibility of refibrillation was assessed after initial long-duration VF by attempting to induce VF. Sustained refibrillation was successful in fewer ranolazine-treated (29.17%; P=0.005) or GS-967–treated (45.83%; P=0.035) hearts compared with that in nontreated control hearts (84.85%). In GS-967–treated hearts, significantly more spontaneous termination of initial long-duration VF was observed (66.67%; P=0.01). Ca2+ transient duration was reduced in ranolazine-treated hearts compared with that in controls (P=0.05) and also Ca2+ alternans (P=0.03).

Conclusions—Late Na+ current inhibition during long-duration VF reduces the susceptibility to subsequent refibrillation, partially by mitigating dysregulation of intracellular Ca2+. These results suggest the potential therapeutic use of ranolazine and GS-967 and call for further testing in cardiac arrest models. (Circ Arrhythm Electrophysiol. 2017;10:e004331. DOI: 10.1161/CIRCEP.116.004331.)

Key Words: calcium transients • cardiac arrest • GS-967 • late sodium current • ranolazine • ventricular fibrillation • ventricular refibrillation

Ventricular fibrillation (VF) is a significant cause of sudden cardiac death.1,2 Despite improvements in cardiopulmonary resuscitation, the survival rate of out-of-hospital cardiac arrest remains poor, in part because of failure to defibrillate and a high incidence of refibrillation after initial defibrillation.3,6 Dysregulation of intracellular Ca2+ dynamics is thought to be an important mechanism in refibrillation after initial defibrillation.7 Recently, we demonstrated that the use of ryanodine receptor 2 blockade as a therapeutic strategy to treat VF is associated with decreased intracellular Ca2+ wave fronts and Ca2+ overload after initial VF.8 Here we pursue an alternate pathway to modulate abnormal intracellular Ca2+ dynamics to prevent refibrillation after long-duration VF (LDVF).

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It has been demonstrated that an enhanced late Na+ current is responsible for the pathological increase in intracellular Na+.9 This drives an efflux of Na+ via the Na+-Ca2+ exchanger, with a corresponding influx of Ca2+ resulting in intracellular Ca2+ overload.10,12 a key step in cardiac arrhythmogenesis.15–16 Previous studies have shown that the late Na+ current is unable to inactivate in the settings of myocardial ischemia and heart failure.9,17,18 Because VF results in massive global ischemia, these ionic derangements originating from the late Na+ current and Ca2+ dynamics may serve as therapeutic targets for preventing ventricular refibrillation after LDVF.

The antianginal drug ranolazine is an inhibitor of the late Na+ current. Earlier studies have demonstrated the antiarrhythmic effect of ranolazine on atrial fibrillation and ventricular tachycardia.19–22 However, the impact of ranolazine on ventricular refibrillation after initial LDVF has not been studied in detail. We hypothesized that ranolazine will mitigate refibrillation after LDVF. Given the recognized inhibition of peak inward Na+ current and inward-rectifier K+ current

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WHAT IS KNOWN

- After defibrillation, it is crucial to prevent refibrillation to ensure successful resuscitation outcomes.
- After ischemia, the late sodium current remains active. Inability of late sodium current to inactivate leads to cardiac arrhythmias.

WHAT THE STUDY ADDS

- Our study demonstrated that the late sodium current plays an important role in maintenance and reinitiation of ventricular fibrillation.
- Blockade of the late sodium current prevents defibrillation and improves defibrillation outcome.
- Inhibition of the late sodium current has antiarrhythmic properties in the ventricle during global ischemia.

in addition to the late Na\(^+\) current at therapeutic concentrations of ranolazine,\(^{21}\) we also sought to further characterize the effect of specific late Na\(^+\) current blockade with a selective inhibitor GS-967.

Methods

Ex Vivo Rabbit Heart Langendorff

New Zealand White rabbits (3–4 kg) were anesthetized with 5% isoflurane, and their hearts were excised through thoracotomy following an animal use protocol approved by the University Health Network Animal Care Committee. Each heart was then immersed in ice-cold modified Tyrode’s solution, containing the following (in mM): NaCl 130, KCl 4.4, CaCl\(_2\) 2.2, MgSO\(_4\) 0.3, NaH\(_2\)PO\(_4\) 1.2, NaHCO\(_3\) 24, and glucose 12 and bubbled with 95% oxygen and 5% CO\(_2\). After the aorta was cannulated, the heart was perfused with modified Tyrode’s solution, with temperature maintained at 37°C and pressure at =70 mm Hg, in a Langendorff setup.

Experimental Protocol

After stabilizing each heart at the Langendorff apparatus, simultaneous dual optical mapping of calcium and voltage signals was performed following a pace and pause protocol. Briefly, rabbit hearts were paced at 5, 5.5, and 6 Hz for 30 s to reach a steady state. Pacing was then halted, and optical mapping was performed at the end of the pacing period. Optical signals were recorded during the last 2 s of pacing and the first 2 s of spontaneous rhythm and were analyzed to assess the action potential duration (APD) and Ca\(^{2+}\) dynamics. Electrical recording and stimulation were performed as described previously.\(^{23}\) Briefly, 4 silver electrodes each 4 mm apart were placed on a plastic seat for the perfused heart; 2 electrodes were used for stimulation, and the other 2 electrodes were used for recording electrical activity from the left ventricular epicardium.

VF was induced using a combination of burst pacing (50 Hz, 12 V, and pulse width of 4 ms) for 10 s and direct contact with a 9 V battery simultaneously to ensure successful induction of initial VF. After confirmation of VF by surface ECG, dual optical mapping was performed at time 0 and was repeated every 2 minutes. Isoproterenol (300 nmol/L) was added to the perfusion buffer during VF to simulate sympathetic hyperactivity. Perfusion was halted immediately after induction of VF to simulate the global ischemia resulting from VF. After 2 minutes of ischemic VF, perfusion resumed with modified Tyrode’s solution added with ranolazine (10 \(\mu\)M; Sigma Aldrich) or GS-967 (0.3 \(\mu\)M; MedChem Express) or the same volume of isotonic saline in the control group. Standard doses of ranolazine and GS-967 were selected based on previous studies.\(^{11,24-26}\) VF was then terminated at 6 minutes using a custom-made external defibrillation pad starting at 5 J energy level. Three minutes after defibrillation, dual optical mapping was performed to measure APD and Ca\(^{2+}\) dynamics following the same pace and pause protocol as described earlier.

Optical Mapping

Rabbit hearts were stained with a Ca\(^{2+}\)-sensitive fluorescent dye, Rhod2-AM (0.2 \(\mu\)M; Biotium, Inc), and a voltage-sensitive dye, RH237 (0.2 \(\mu\)mol/L; Biotium, Inc). To remove motion artifacts, we infused a mechanical uncoupler, blebbistatin (1 \(\mu\)mol/L; Enzo Life Sciences, Inc). The detailed methodology for the Langendorff setup and optical mapping has been described elsewhere.\(^{8}\)

Phase Mapping and Spatial Organization During VF

As a surrogate for analyzing spatial organization of VF, phase mapping of Ca\(^{2+}\) and voltage recordings was performed as previously described.\(^{4}\) Optical mapping data obtained during VF episodes were analyzed to assess spatial organization of VF under control versus ranolazine-treated conditions. A 4-s sequence of optical data was first detrended to remove baseline wander and bleach curve using a first-order polynomial fitting, followed by Hilbert phase transform. Waves were identified by localizing contiguous areas where the phase value corresponded to phase 0 of depolarization. Waves that were at least 25 pixels long were included in the analysis. The total number of waves included the waves from both Ca\(^{2+}\) and voltage data observed during the 4-s VF segment. Data from 3 experiments (1 control and 2 ranolazine) were not included in this analysis because VF spontaneously terminated before the study time point or because of poor optical signals.

Voltage and Ca\(^{2+}\) Signal Feature Analysis

Left ventricular epicardial Ca\(^{2+}\) and action potential fluorescence were mapped simultaneously after initial LDVF to determine the effects of ranolazine and GS-967 on Ca\(^{2+}\) and action potential dynamics. Four seconds of optical data were recorded at the end of the 30-s pacing and included 1 or more spontaneous beats post-stimulation. One pixel from an area on the optical maps representing the left ventricle was selected for a feature analysis of voltage and Ca\(^{2+}\) signals. Voltage and Ca\(^{2+}\) mapping data from 2 hearts were excluded because of poor quality of optical signals. The following parameters were studied:

1. APD50 and APD80 measurements: APD measured from 0% to 50% repolarization (APD50) and from 0% to 80% repolarization (APD80) were calculated for each beat during the last 2 s of pacing rhythm. Eighty percent and 50% repolarization were referenced from the peak of the action potential to the baseline measured before the action potential takeoff.
2. CaTD50 and CaTD80 measurements: Ca\(^{2+}\) transient durations (CaTD) measured from 0% to 50% repolarization (CaTD50) and from 0% to 80% repolarization (CaTD80) were also calculated for each beat during the last 2 s of pacing rhythm, with similar signal processing to APD50 and APD80.
3. Ca\(^{2+}\) Amplitude alternans ratio: Ca\(^{2+}\) amplitude alternans ratio was calculated as \(y/x\), where \(x\) is the amplitude of smaller and \(y\) is the amplitude of larger Ca\(^{2+}\) transient. Thus, a ratio of 1 is indicative of no alternans. The alternans ratio was measured during pacing at a rate of 5, 5.5, or 6 Hz after initial LDVF and compared between ranolazine- and GS-967–treated and nontreated control hearts.

4. Spontaneous diastolic Ca\(^{2+}\) elevation: Spontaneous diastolic Ca\(^{2+}\) elevation was measured using a method adopted from Lee et al.\(^{22}\) Briefly, diastolic Ca\(^{2+}\) elevation was measured before the first spontaneous beat after the 30-s stimulation and normalized to Ca\(^{2+}\) amplitude during pacing at different rates and expressed as an arbitrary unit (AU). All signal processing in this study was performed using Matlab 2013 (MathWorks).
Susceptibility to Induction of Subsequent VF (Refibrillation)

Reinducibility and sustainability of VF were assessed in ranolazine- or GS-967–treated and nontreated control hearts by burst pacing the hearts in the postdefibrillation period at 50 Hz and 12 V for 10 s. Successfully induced VF was defined as VF lasting ≥10 s, and sustained VF was defined as VF lasting ≥60 s. All hearts were defibrillated after 1 minute of VF, and VF induction was repeated for a total of 4× during the postdefibrillation period, with 3 minutes recovery between each attempt. The refibrillation data from 1 heart was not included because of corrupted electrical data.

Statistical Analysis

Continuous data are presented as means±SE. Comparisons of calcium and voltage wave front count between the 2 groups (control versus ranolazine) were performed using Mann–Whitney test. For CaTD, APD, Ca$^{2+}$ alternans, and spontaneous diastolic Ca$^{2+}$ elevation at different pacing rates, nonparametric Kruskal–Wallis test followed by multiple pairwise comparison using Dunn’s post test was used to compare these variables across the 3 groups (control versus ranolazine versus GS-967). Repeated measure logistic regression analysis using the Xggee command in Stata, which fits population-averaged panel data models by using generalized estimating equations (fits generalized linear models), was used to compare the inducibility and sustainability of consecutive ventricular refibrillation episodes among the ranolazine- or GS-967–treated and control groups. For the spontaneous termination of VF analysis, we used effect measures by calculating the absolute risk reduction. A P value of ≤0.05 is considered to be statistically significant. All statistical analyses were performed using Stata 11.2 (Stata Corp LP), SPSS 17, and GraphPad Prism 5.

Results

Effects of Ranolazine and GS-967 on VF and Refibrillation

Up to 4 episodes of ventricular refibrillation were attempted on each heart after the initial LDVF. Successful induction of sustained refibrillation (VF lasting ≥60 s) was achieved 84.8% in nontreated controls, 29.2% in ranolazine-treated hearts (P=0.005 compared with nontreated control), and 45.8% in GS-967–treated hearts (P=0.035 compared with nontreated controls; Table 1). Induction of refibrillation defined as lasting ≥10 s was achieved in 87.9% of nontreated controls as compared with 33.3% among ranolazine-treated (P=0.006 compared with nontreated controls) and 70.8% of GS-967–treated groups (P=0.26 compared with nontreated control; Table 1). In ranolazine-treated hearts, LDVF electroggrams resembled monomorphic or polymorphic ventricular tachycardia compared with those in nontreated control hearts (Figure 1).

The median number of successful induction of sustained ventricular refibrillation episodes in the ranolazine-treated, as well as in the GS-967–treated, groups was significantly lower than that in the nontreated group (Figure 2A). The median number of refibrillation episodes lasting ≥10 s was significantly lower in the ranolazine-treated group compared with that in the nontreated group.

Spontaneous termination of the initial LDVF occurred significantly more frequently in the GS-967–treated hearts (66.7%) as compared with that in ranolazine-treated (14.3%) and nontreated control (11.1%) hearts (Figure 2B). The absolute benefit increase for the GS-967–treated group compared with the nontreated control group was 0.55 (95% confidence interval, 0.13–0.99; P=0.011). The spontaneous termination of refibrillation occurred within 25 s in the majority of cases in the GS-967–treated group.

Effects of Ranolazine on Spatial Organization of LDVF

In several rabbit hearts, LDVF transitioned to a more organized rhythm after treatment with ranolazine and spontaneously terminated in GS-967–treated heart. Spatial organization of VF was compared using phase maps of Ca$^{2+}$ and voltage signals acquired during VF at time 0 (immediately after induction of VF) and at 4 minutes. The number of wave fronts was used as a surrogate of spatial organization of VF in rabbit hearts. At time 0 of VF, there was no statistically significant difference in the mean number of Ca$^{2+}$ wave fronts between ranolazine-treated hearts and nontreated controls (Figure 3A and Table 2). In our analysis of VF that did not terminate, with regards to mean number of Ca$^{2+}$ wave fronts in the ranolazine-treated hearts at 4 minutes of VF, we did not see statistically significant decrease when compared with nontreated control group (P=0.13; Figure 3A and 3B and Table 2; Movie I in the Data Supplement). However, in VF episodes that did not terminate, there was borderline increase in the number of wave fronts in the ranolazine-treated groups (P=0.05; Table 2) as assessed by action potential mapping. Evaluating the effect of GS-967 on spatial organization of LDVF was not possible because VF spontaneously terminated frequently before the 4-minute time point used for this analysis, as detailed in the earlier section.

Effects of Ranolazine and GS-967 on CaTD and APD After LDVF

Left ventricular epicardial Ca$^{2+}$ and action potential fluorescence were mapped simultaneously after initial LDVF to determine the effects of ranolazine and GS-967 on APD and Ca$^{2+}$ dynamics. The mean CaTD80 was significantly shorter in the GS-967–treated hearts (P=0.05 at 5 Hz compared with that in nontreated control hearts (Figure 4A) without any changes in CaTD50 (Figure 4). There were no significant differences in APD80 and APD50 between ranolazine-treated, GS-967–treated, and nontreated hearts at various cycle lengths (Figure 5).

<table>
<thead>
<tr>
<th>Table 1. Ventricular Refibrillation Vulnerability After LDVF</th>
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<tr>
<td>Control, % (n=9)</td>
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<td>------------------</td>
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<tr>
<td>Sustained Refibrillation ≥60 s</td>
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<tr>
<td>Refibrillation lasting ≥10 s</td>
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Summary of results showing the susceptibility of sustained ventricular refibrillation lasting ≥60 s and ventricular refibrillation lasting ≥10 s between ranolazine, GS-967–treated, and nontreated control hearts. LDVF indicates long duration ventricular fibrillation.

*P=0.005. †P=0.035. ‡P=0.006 compared with nontreated controls by repeated measures logistic regression analysis.
Effects of Ranolazine and GS-967 on Ca²⁺ Amplitude Alternans and Spontaneous Ca²⁺ Leak

We analyzed the Ca²⁺ amplitude alternans ratios during pacing at various cycle lengths. At 5 Hz, Ca²⁺ amplitude alternans were significantly less in ranolazine-treated heart compared with those in GS-967–treated heart (P=0.03; Figure 6A). There were also significantly less Ca²⁺ amplitude alternans in ranolazine-treated hearts compared with those in nontreated controls at 6 Hz (P=0.03; Figure 6C).

There were no significant differences in the amplitude of the spontaneous diastolic Ca²⁺ elevation between ranolazine- or GS-967–treated groups at any of the pacing rates.
tested compared with that in nontreated controls (data not shown).

**Discussion**

We demonstrated decreased susceptibility of sustained ventricular fibrillations in both ranolazine-treated and GS-967–treated hearts compared with that in nontreated controls after initial LDVF in an ex vivo rabbit heart model. Furthermore, we observed improved calcium alternans and shortened CaTDs in the ranolazine-treated hearts. Moreover, there was greater spontaneous defibrillation of the VF in the GS-967–treated group from sustained VF. Our findings in optical mapping provide mechanistic insights to the use of this late Na+ current inhibitor during LDVF partially on the basis of mitigating disordered calcium dynamics while not affecting action potential dynamics on the epicardium. These findings are consistent with the evolving evidence that suggest a role for the late sodium current and ranolazine in the modulation of ventricular arrhythmias.20,21,30

**Late Na+ Current Blockade and VF and Refibrillation**

In our experimental model, ranolazine treatment beginning after 2 minutes of ischemic VF did not result in spontaneous termination of VF. In contrast, treatment with GS-967, a specific inhibitor of the late Na+ current, led to a significant increase in spontaneous termination of VF. In an earlier study, Morita et al31 demonstrated spontaneous defibrillation of the VF in the GS-967–treated group from sustained VF. Our findings in optical mapping provide mechanistic insights to the use of this late Na+ current inhibitor during LDVF partially on the basis of mitigating disordered calcium dynamics while not affecting action potential dynamics on the epicardium. These findings are consistent with the evolving evidence that suggest a role for the late sodium current and ranolazine in the modulation of ventricular arrhythmias.20,21,30

![Figure 3. Effects of ranolazine on ventricular fibrillation (VF) organization. A, Tracings of Ca2+ signal at 0 minutes (before ranolazine/saline treatment) and at 4 minutes of VF (with ranolazine/saline treatment). B, Representative images of phase maps of Ca2+ wave fronts at 4 minutes of VF, with arrows indicating the wave fronts.](image)

**Table 2. Calcium and Voltage Wavefront Count During LDVF**

<table>
<thead>
<tr>
<th></th>
<th>Control (n=8)</th>
<th>Ranolazine (n=5)</th>
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<tbody>
<tr>
<td><strong>Ca2+ wave fronts</strong></td>
<td></td>
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<tr>
<td>At 0 min</td>
<td>2.52±0.23</td>
<td>2.60±0.15</td>
</tr>
<tr>
<td>At 4 min</td>
<td>2.80±0.16</td>
<td>2.15±0.34</td>
</tr>
<tr>
<td><strong>Voltage wave fronts</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At 0 min</td>
<td>3.70±0.25</td>
<td>4.10±0.14</td>
</tr>
<tr>
<td>At 4 min</td>
<td>4.23±0.15</td>
<td>4.90±0.29*</td>
</tr>
</tbody>
</table>

Mean number of Ca2+ and voltage wave fronts per frame at 0 min (before treatment) and at 4 min (after treatment) of LDVF in ranolazine-treated hearts and in nontreated controls. LDVF indicates long duration ventricular fibrillation. *P=0.05 compared with nontreated controls at 4 min by Mann–Whitney test.
reduction in ischemia-induced atrial and ventricular repolarization alternans and ECG heterogeneity. Similarly, Justo et al.\textsuperscript{34} selectively inhibited the late Na\textsuperscript{+} current with the drug eleclazine and found that it reduced ventricular repolarization abnormalities without reducing inotropy before and during adrenergic stimulation with epinephrine. Our study further supports that the late Na\textsuperscript{+} current plays an important role in the maintenance and reinitiation of VF, and its blockade may both prevent refibrillation and improve defibrillation outcome. The differences in efficacy between ranolazine and GS-967 for spontaneously terminating VF could be accounted for by differences in the specificity of late Na\textsuperscript{+} current blockade, with the former also exerting effects on peak Na\textsuperscript{+} current, inward-rectifier K\textsuperscript{+} channels, and improvement of mitochondrial function, or could be because of small sample size.

**Figure 4.** Effects of ranolazine and GS-967 on Ca\textsuperscript{2+} dynamics after long-duration ventricular fibrillation (LDVF). A, Ca\textsuperscript{2+} transient duration CaTDT80 at 5 Hz; (B) Ca\textsuperscript{2+} transient duration CaTDT80 at 5.5 Hz; (C) Ca\textsuperscript{2+} transient duration CaTDT80 at 6 Hz; (D) CaTDT50 at 5 Hz; (E) CaTDT50 at 5.5 Hz; and (F) CaTDT50 at 6 Hz between ranolazine-treated group (n=5), GS-967-treated groups (n=6), and nontreated controls (n=9). *P<0.05 compared with nontreated controls at 5 Hz by Kruskal–Wallis test followed by multiple pairwise comparison using Dunn's post test.

**Ranolazine, GS-967, and Intracellular Ca\textsuperscript{2+} Dynamics After LDVF**

The administration of ranolazine during LDVF led to shortened CaTDS and reduced Ca\textsuperscript{2+} alternans after the LDVF. Ca\textsuperscript{2+} amplitude alternans reflects changing intracellular Ca\textsuperscript{2+} dynamics and is associated with cardiac arrhythmogenesis,\textsuperscript{35} and our recent study demonstrated that highly organized VF is associated with fewer Ca\textsuperscript{2+} wave fronts.\textsuperscript{3} In the present study, we observed LDVF return to sinus rhythm spontaneously by inhibition of the late sodium current. A previous study by Ogawa et al.\textsuperscript{7} demonstrated that elevation of persistent Ca\textsuperscript{2+} during late phase 3 and phase 4 of action potential triggered recurrences of spontaneous VF. Consistent with this, in our study, significant reduction of CaTDT80 after LDVF in ranolazine-treated hearts was associated with the reduced occurrence of ventricular refibrillation. There are also data from prior studies on the reduction of atrial fibrillation with late Na\textsuperscript{+} current blockade.\textsuperscript{34,36} The induction and maintenance of atrial fibrillation are similarly affected by intracellular Ca\textsuperscript{2+} dynamics, further supporting the mechanistic hypothesis that the inhibition of the late Na\textsuperscript{+} current may modulate arrhythmogenesis by improving Ca\textsuperscript{2+} dynamics. In our study, the use of GS-967 did not lead to significant reductions of Ca\textsuperscript{2+} transient or Ca\textsuperscript{2+} amplitude alternans after initial LDVF, but we observed spontaneous termination of initial VF in GS-967-treated hearts.

We observed reduction in the Ca\textsuperscript{2+} alternans in the ranolazine-treated group and not in the GS-967–treated hearts. One possible explanation could be the effects of ranolazine on mitochondrial function. Metabolic changes in the myocardial tissue during ischemia because of uncoupling of mitochondria is a proarrhythmic substrate for development of ventricular arrhythmias.\textsuperscript{27} It has been demonstrated that, after ischemia, ranolazine improves mitochondrial function and that ranolazine is also protective of mitochondria in ischemia reperfusion injury.\textsuperscript{37–39} In a previous study, we have demonstrated that improvement of mitochondrial function is cardioprotective.\textsuperscript{40} This may support the notion that ranolazine improves mitochondrial function after LDVF. Therefore, it is likely that ranolazine preserved the mitochondrial function during the ischemic phase of VF, improving the ATP-dependent intracellular Ca\textsuperscript{2+} dynamics. Data are presently lacking on the impact of GS-967 on mitochondrial function in ischemia. Our observation that GS-967 did not improve Ca\textsuperscript{2+} dynamics may be explained by differential effects in the protective effects on mitochondria between GS-967 and ranolazine.

In our study, there were no significant differences in the diastolic Ca\textsuperscript{2+} leak on the epicardium between the 3 groups. However, an earlier study by Undrovinas et al.\textsuperscript{25} showed that in chronic heart failure, late Na\textsuperscript{+} current contributes to diastolic Ca\textsuperscript{2+} leak and that ranolazine inhibits diastolic Ca\textsuperscript{2+} leak.

**Late Na\textsuperscript{+} Current Blockade and APD After LDVF**

We did not observe significant differences in APD between ranolazine-treated, GS-967–treated, and nontreated control hearts after LDVF. Data regarding the effect of ranolazine on APD in the published literature have been conflicting, with some studies demonstrating the prolongation of APD in the presence of ranolazine in both normal and failing hearts,\textsuperscript{41,42} whereas a study by
Antzelevitch et al. showed that APD was indeed shortened after ranolazine treatment in canine ventricular M cells and Purkinje fibers but prolonged in ventricular epicardium. Ranolazine also inhibited APD prolongation in patients with type 3 long QT syndrome. Further work by Morita et al. demonstrated that ranolazine has no effect on APD. The contradictory observations on the effects of ranolazine on APD suggest that results are highly dependent on experimental conditions, as well as on the tissues studied.

Limitations
One significant limitation of any Langendorff setup is the denervated nature of the isolated hearts, which lack sympathetic and parasympathetic regulation of the myocardium that may result in different ex vivo outcomes compared with in vivo VF models. However, we have previously shown that the VF dynamics in Langendorff-based study are similar to the VF dynamics in ambulatory humans. Our recordings were limited to the epicardial surface of
rabbit hearts, and further study is required before generalizing our findings to other animal and human hearts. Spontaneous reinitiation of VF was not specifically studied because of the rarity of its occurrence in our denervated explanted heart model. However, we have indeed observed spontaneous termination of initial VF with administration of GS-967, which is highly relevant clinically.

Conclusion
Treatment with late Na\(^+\) current blockade during LDVF decreases susceptibility to subsequent ventricular refibrillation and facilitates defibrillation of LDVF by partially mitigating dysregulation of intracellular Ca\(^{2+}\) dynamics. The present experimental findings form the basis for further studies in cardiac arrest models to evaluate the therapeutic potential of ranolazine and GS-967 in VF arrest to prevent refibrillation and facilitate defibrillation.

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Disclosures
None.

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