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Suppression of Re-Entrant and Multifocal Ventricular Fibrillation by the Late Sodium Current Blocker Ranolazine

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Objectives The purpose of this study was to test the hypothesis that the late Na current blocker ranolazine suppresses re-entrant and multifocal ventricular fibrillation (VF).

Background VF can be caused by either re-entrant or focal mechanism.

Methods Simultaneous voltage and intracellular Ca^{+2} optical mapping of the left ventricular epicardial surface along with microelectrode recordings was performed in 24 isolated-perfused aged rat hearts. Re-entrant VF was induced by rapid pacing and multifocal VF by exposure to oxidative stress with 0.1 mM hydrogen peroxide (H_2O_2).

Results Rapid pacing induced sustained VF in 7 of 8 aged rat hearts, characterized by 2 to 4 broad propagating wavefronts. Ranolazine significantly ($p < 0.05$) reduced the maximum slope of action potential duration restitution curve and converted sustained to nonsustained VF lasting 24 ± 8 s in all 7 hearts. Exposure to H_2O_2 initiated early afterdepolarization (EAD)-mediated triggered activity that led to sustained VF in 8 out of 8 aged hearts. VF was characterized by multiple foci, appearing at an average of 6.8 ± 3.2 every 100 ms, which remained confined to a small area averaging 2.8 ± 0.85 mm^2 and became extinct after a mean of 43 ± 16 ms. Ranolazine prevented (when given before H_2O_2) and suppressed H_2O_2-mediated EADs by reducing the number of foci, causing VF to terminate in 8 out of 8 hearts. Simulations in 2-dimensional tissue with EAD-mediated multifocal VF showed progressive reduction in the number of foci and VF termination by blocking the late Na current.

Conclusions Late Na current blockade with ranolazine is effective at suppressing both pacing-induced re-entrant VF and EAD-mediated multifocal VF. (J Am Coll Cardiol 2011;57:366–75) © 2011 by the American College of Cardiology Foundation

Pharmacologic and genetic approaches to treatment of ventricular fibrillation (VF) must take into account its mechanistic complexity. VF can be caused by both re-entrant and focal mechanisms, as well as mixtures of the 2. Rapid pacing-induced VF generally is attributed to re-entrant mechanisms, resulting from either multiple wavelets (1) or a mother rotor (2), depending on experimental conditions (3). However, drugs and genetic defects that reduce repolarization reserve (prolong repolarization) and alter intracellular Ca^{+2} (Ca_i^{2+}) cycling promote polymorphic ventricular arrhythmias degenerating to VF, in which focal mechanisms are involved in VF initiation and maintenance. For example, local application of aconitine, which impairs Na current inactivation, induces a form of VF that is maintained by focal activity originating from the site of aconitine application (4,5). Multifocal VF also can be induced by oxidative stress with hydrogen peroxide (H_2O_2), which is driven by early afterdepolarization (EAD)-mediated triggered activity generating multiple short-lived foci continuously shifting in location (6,7).

Ranolazine, which preferentially blocks the late Na current (I_{Na-L}) (8,9), is a clinically useful antianginal drug that has been shown to exert antiarrhythmic actions as well (10). In this respect, ranolazine is shown to reduce the dispersion of ventricular action potential duration (APD) (11–13), an effect

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that may account for the drug's demonstrated efficacy to increase VF threshold and decrease ventricular defibrillation threshold of re-entrant VF. (14) Ranolazine also has been shown to suppress EADs and triggered activity in isolated cardiac myocytes exposed to H_2O_2 (8,15); however, its effects on the initiation and maintenance of EAD-mediated multifocal oxidative VF in intact hearts have not been evaluated. H_2O_2 has pleiotropic effects, including direct effects on ion channel and transporter proteins and indirect effects mediated by activating Ca-calmodulin kinase II signaling pathways (16–18), which enhance the late Na current, promoting EADs and triggered activity (8,15,19). The aim of this study was to compare the effects of ranolazine on pacing induced re-entrant VF and on H_2O_2-induced multifocal VF.

Methods

The research protocol was approved by the Institutional Animal Care and Use Committee and followed the guidelines of American Heart Association.

Langendorff setting. We used male Fisher344 rats age 24 to 26 months (n = 24) purchased from the National Institute on Ageing, Bethesda, Maryland. The hearts of the anesthetized rats were removed and the ascending aorta was cannulated for retrograde perfusion with warm (36.5 ± 0.50°C) oxygenated Tyrode's solution, as described previously (6,20).

Optical mapping. The hearts were stained with RH237 and Rhod-2 AM (Invitrogen Molecular Probes, Carlsbad, California) for simultaneous dual voltage and Ca_i^{2+} fluorescent optical imaging, respectively, as described previously (6). Ca_i^{2+} transient decay rate constant, τ, was determined by a monoexponential fit during the relaxation before and after ranolazine (6). Cytochalasin D (5 μM) was added to the perfusate to inhibit motion (6). Single-cell action potentials were recorded with glass microelectrodes from the base of the left ventricle (LV) epicardium, the site of the focal activity during the onset of VT as determined by optical mapping (6). An epicardial wavefront was considered to be focal when it arose from within the mapped region (i.e., did not propagate into the mapped region from the outside) and was surrounded with nonexcited tissue. We previously showed that after extensive endomyocardial and midmyocardial cryoablation, the epicardial surface cells generate focal activity independent of breakthrough excitation from deeper cells (6).

Dynamic APD restitution. Epicardial APD was measured at 90% and 50% repolarization from multiple epicardial cells (3 to 5 cells) recorded with microelectrodes from base mid and apical regions in 8 hearts before and after ranolazine. APD restitution curves were determined using a dynamic pacing protocol, as described previously (6,20).

Pharmacological interventions. The isolated hearts first were perfused with normal Tyrode's solution, and baseline pacing-induced arrhythmias were recorded and imaged. Then, the effect of ranolazine (10 μM) on inducible VF was evaluated 20 min after perfusion (n = 8). When the VF lasted longer than 3 min, it was defibrillated by electrical shock. In a separate series of experiments, the effect of ranolazine on H_2O_2 (0.1 mM)-initiated VF was evaluated. This involved 2 protocols. The purpose of the first protocol was to determine the efficacy of ranolazine to suppress the VF after it is initiated in the continuous presence of H_2O_2 (n = 8). The purpose of the second protocol was to test the efficacy of ranolazine to prevent the emergence of VF when administered 20 min before H_2O_2 (0.1 mM, n = 8).

Abbreviations and Acronyms

2D = 2-dimensional
APD = action potential duration
Ca_i^{2+} = intracellular calcium ion transient
dV/Dt_{max} = maximum rate of phase-zero action potential depolarization
EAD = early afterdepolarization
H_2O_2 = hydrogen peroxide
$I_{Na\text{-}L}$ = late component of the fast sodium inward current
LV = left ventricle
VF = ventricular fibrillation
VT = ventricular tachycardia

2-dimensional computer simulation studies. Computer simulations were performed in a 2-dimensional (2D) tissue model with the membrane voltage described by the following partial differential equation:

$$\frac{\partial V}{\partial t} = -I_{ion}/C_m + D\left(\frac{\partial^2 V}{\partial x^2} + \frac{\partial^2 V}{\partial y^2}\right) \quad [1]$$

where C_m is the membrane capacitance set at 1μF/cm^2, I_{ion} is the total membrane ionic currents, and D is the diffusion coefficient and was set as 0.0005 cm^2/ms with no-flux boundary conditions. I_{stim} is the stimulation current (a square pulse with a strength 40 μF/cm^2 and duration of 1 ms). We used the ventricular myocyte action potential model that we developed recently (21). The model was modified to generate EADs with detailed changes as described previously (7). To model the effects of H_2O_2, in addition to increasing the maximum conductance of I_{CaL} by 2.6 and decreasing the maximum conductance of the rapid delayed rectified potassium current (I_{Kr}) by 20%, we also halved the rate of calcium uptake by the sarcoplasmic reticulum and added $I_{Na\text{-}L}$ that was equivalent to 0.1% of the peak I_{Na} amplitude (7). The effects of ranolazine were simulated by decreasing the late I_{Na}. We first paced a single myocyte with a square pulse (I_{stim} described above) to the steady-state and used it as the initial condition for the myocytes in the whole tissue to save computer time. The 2D tissue then was paced from the corner with 1 beat to allow EAD-mediated focal VF to develop. After 1 min, the dynamics of the focal VF-like state were analyzed in the presence and absence of $I_{Na\text{-}L}$ to study ranolazine effect. Numerically, Equation 1 was discretized with $\Delta x = \Delta y = 0.015$ cm and was integrated with a forward Euler method with an adaptive time step varying from 0.01 to 0.1 ms.