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The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

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RESEARCH CORRESPONDENCE

Rapid Assessment of Proarrhythmic Potential Using Human iPSC-Derived Cardiomyocytes

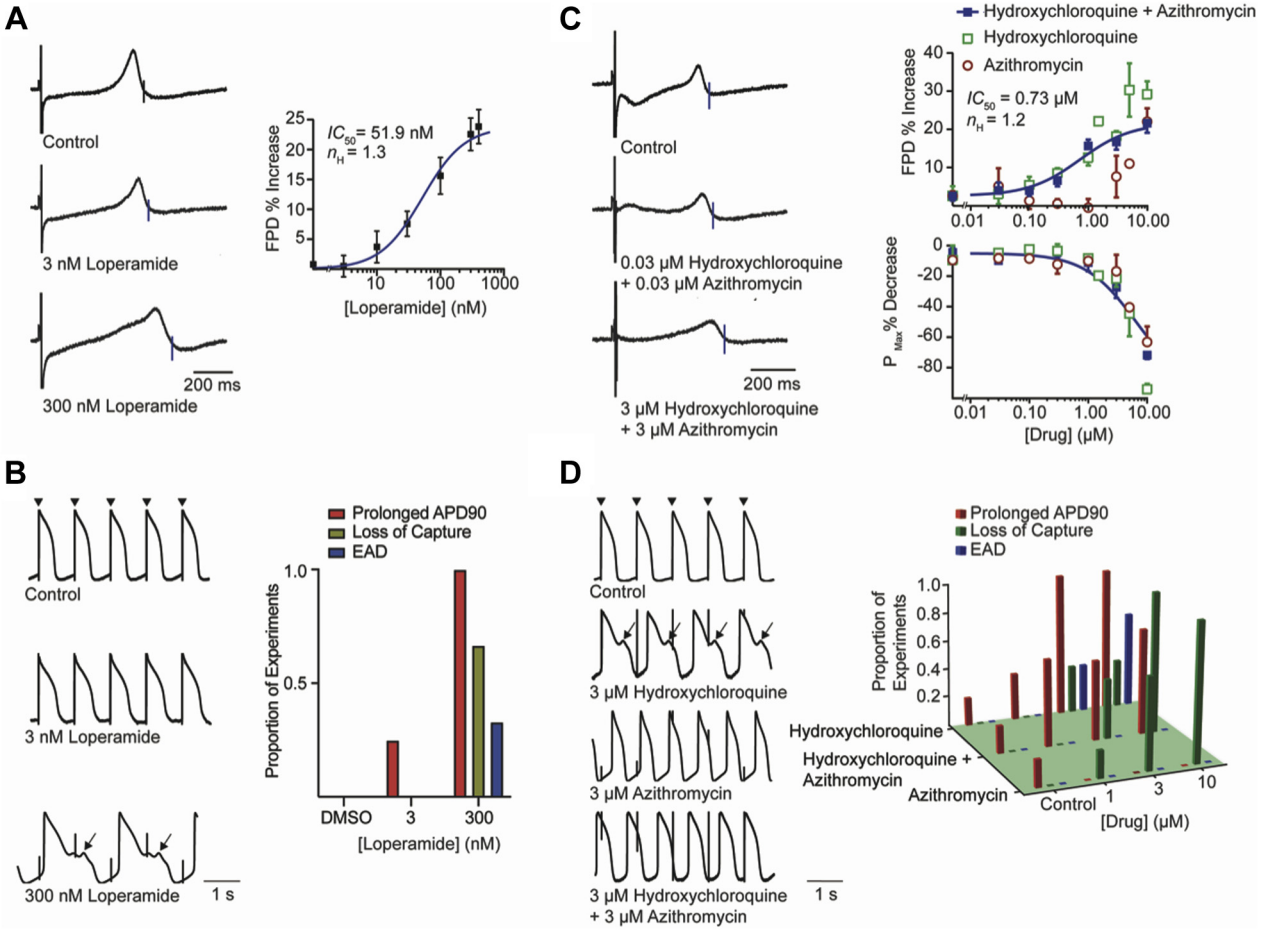
Public health threats like the ongoing epidemics of opioid abuse and COVID-19 can result in restriction of existing drugs or their repurposing in new and potentially dangerous ways. For example, ingestion of high doses of loperamide, an over-the-counter μ-opioid agonist, has increased as opioid-dependent patients seek inexpensive, widely available alternatives in response to stringent prescription drug monitoring programs. Similarly, the use of hydroxychloroquine alone or in combination with azithromycin has been touted as a treatment for SARS-CoV-2, despite an apparent lack of therapeutic benefit (1). Because these drugs are not being used as intended (loperamide) or used in combinations that have not been well studied (hydroxychloroquine and azithromycin), there is little cardiac safety data on these drug and dosing regimens. Importantly, the ability to perform randomized control trials requires time that may be in short supply during an epidemic. Developing approaches to rapidly assess cardiac rhythm stability in a controlled manner are needed to provide timely guidance to ensure patient safety.

To address this need, we examined electrical activity in human-induced pluripotent stem cell (iPSC)–derived cardiomyocyte monolayers using the Axion Maestro Microelectrode Array (MEA) system (Axion Biosystems, Atlanta, Georgia) to rapidly assess potential adverse drug effects on cardiac excitability. All studies were performed on commercially available iPSC-derived cardiomyocytes (iCell^2; Cellular Dynamics, Madison, Wisconsin) and did not require institutional review board approval. Cardiomyocytes were thawed and plated as monolayers on fibronectin-coated MEA plates (24 well; 70,000 cells/well) and cultured for 5 to 12 days before recording. The extracellular field potential duration and amplitude of the early spike (P_{Max}) reflect the mean action potential duration and sodium (Na) conductance, respectively, analogous to the QT interval and QRS complex assessed in a surface electrocardiogram. We also measured the local extracellular action potential (LEAP) that resembles the monophasic action potential. In all experiments, cells were maintained at 37 °C and paced by field stimulation at 1 Hz.

During the ongoing opioid epidemic, a growing number of individuals have presented to emergency departments with QT prolongation and Torsade de pointes associated with ingesting supratherapeutic doses of loperamide. Loperamide, marketed primarily in the United States as Imodium (Johnson & Johnson, New Brunswick, New Jersey), is a peripherally acting μ-opioid agonist used to treat diarrhea. We previously demonstrated that loperamide is a potent inhibitor of the human cardiac ether a` go-go-related gene (hERG), encoding the rapid delayed-rectifier potassium-current (I_{Kr}), which increases the QT-interval duration and risk of Torsade de pointes (2). When applied to iPSC-derived cardiomyocytes (2 to 5 h) **Figure1A**), loperamide caused a concentration-dependent increase in the FPD (50 % Inhibitory concentration [IC_{50}] = 51.9 nM). Moreover, after prolonged exposure (24 h) (**Figure 1B**), higher doses resulted in the emergence of distinct markers of proarrhythmic electrical activity in LEAP recordings, manifested as AP prolongation (APD_{90}) by >10% (Fridericia correction), early afterdepolarizations (EADs) and noncapture by pacing stimuli. Arrhythmia risk markers rarely emerged at the 3-nM concentration, which is near the expected therapeutic serum concentration (< 2 ng/ml), but frequent induction of rhythm instability was observed at supratherapeutic concentrations (300 nM), corroborating mounting cardiac safety concerns for loperamide during the opioid crisis.

In the face of the COVID-19 pandemic, repurposing of hydroxychloroquine, an antimalarial drug, and azithromycin, a macrolide antibiotic, was promoted as a treatment strategy to combat the disease. Like loperamide, hydroxychloroquine is a multichannel inhibitor, particularly of I_{Kr} (3) and would be expected to prolong the QT interval. However, in the setting of

FIGURE 1 Evaluating Proarrhythmic Potential of Novel Drug Regimens Using Human iPSC-Derived Cardiomyocytes



(A) Representative field potentials recorded from induced pluripotent stem cell (iPSC)–derived cardiomyocyte monolayers before and 2 to 5 h after loperamide exposure. Field potential duration (FPD) was measured from the initial depolarization spike (analogous to the QRS complex) to the end of the T-wave **(vertical line)**. FPD was unaffected at therapeutic (3 nM) concentrations but markedly increased at supratherapeutic (300 nM) concentrations. Data points in the dose–response curve represent means ± SEM (n = 4 to 7). Curves represent the least-squares regression fit of the data with a Hill equation (Hill coefficient = 1.3). **(B)** Exemplar local extracellular action potentials (LEAPs) from the same cells recorded after 18 to 25 h of exposure to loperamide. At supratherapeutic concentrations, LEAP recordings revealed proarrhythmic activity, including >10% prolongation of the action potential duration (APD) **(red)**, early afterdepolarizations (EADs) **(arrows, blue bar)**, and failure of stimulus capture **(green)**. The bar graph represents the proportion of wells exhibiting the indicated proarrhythmic phenotype (n = 3 to 4). **(C)** Field potential recordings and dose-response curves illustrating FPD prolongation and reduction of amplitude of early field potential spike (P_{max}), respectively, in cardiomyocytes exposed to increasing concentrations of hydroxychloroquine and azithromycin. Dose–response curves represent the least-squares fit of the data with a Hill equation (Hill coefficient = 1.2) (n = 4 to 8). **(D)** Sample LEAP records from cells treated with either hydroxychloroquine, azithromycin, or both, in combination. Summary results illustrate the frequency of monolayers exhibiting each identified proarrhythmic activity (EADs: **blue**; APD prolongation: **red**; loss of capture: **green**) with each of the treatments. APD prolongation and loss of stimulus capture were evident in cells treated with both hydroxychloroquine and azithromycin; however, EADs were absent, which suggested a complex interplay of the proarrhythmic consequences of the combined therapy (n = 3 to 8). All recordings were analyzed with the cardiac module of the Maestro Pro software (Axiom Biosystems, Atlanta, Georgia) or custom software programmed in Python or IDL.

a multidrug regimen, concurrent blockade of depolarizing currents may mitigate these effects (4). Azithromycin inhibits I_{Na} and I_{Ca}, with only low affinity blockade of I_{Kr}, whereas long-term exposure can increase I_{Na} (5). Thus, the net effect of this drug combination on cardiac excitability is difficult to predict but can be rapidly tested using iPSC-derived cardiomyocytes.

Exposure to equal concentrations of both hydroxychloroquine and azithromycin increased FPD in a concentration-dependent manner (IC_{50} = 0.73 μM) (**Figure 1C**). Hydroxychloroquine alone prolonged the

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FPD over a similar range of concentrations, as did azithromycin, but with 4-fold lower sensitivity. Although hydroxychloroquine-dependent block of potassium channels received the most attention, we also observed a decrease in the depolarization spike amplitude by hydroxychloroquine and azithromycin (P_{Max}) (**Figure 1C**), which suggested that both drugs also inhibit depolarizing I_{Na} or I_{Ca} equally. Proarrhythmic activity was also observed in LEAP recordings during prolonged exposure (**Figure 1D**). Exposure to hydroxychloroquine alone caused APD_{90} prolongation, frequent EADs, and loss of stimulus capture, whereas azithromycin caused a loss of stimulus capture with an increase in the intrinsic beat rate. However, EADs were not observed with azithromycin, either alone or in combination with hydroxychloroquine. These results suggested that this specific drug combination should be restricted to patients undergoing electrocardiographic monitoring.

The present study demonstrated the feasibility and usefulness of using iPSC-derived cardiomyocytes to rapidly assess and stratify proarrhythmic risks of drugs that are abused (e.g., loperamide) or applied in uncharacterized combinations (e.g., hydroxychloroquine and azithromycin). This novel approach to evaluating pharmacodynamic effects on cardiac rhythm stability has the potential to rapidly identify clinical risks associated with diverse treatment strategies.

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TO THE EDITOR

J-Wave Syndromes

Where's the Scar?

We read with great interest the paper by Boukens et al. (1) detailing the pathophysiology underlying inferior early repolarization (ER) pattern in a patient with ventricular fibrillation storms and early repolarization syndrome (ERS). Open chest epicardial mapping demonstrated delayed activation and heterogeneous repolarization of the inferior right ventricular free wall and fractionated local potentials coinciding with the electrocardiographic (ECG) ER pattern, which were successfully treated by radiofrequency ablation. Most interestingly, morphometric analysis of biopsied cardiac tissue from this region showed areas of extensive fibrosis. This study provides the first in vivo evidence that the ER pattern can be the ECG manifestation of temporal heterogeneity in cardiac activation due to regional replacement fibrosis.

ERS and the Brugada syndrome (BrS) are characterized by J-point elevation (J-wave syndromes), which both predispose to sudden cardiac death (SCD) in young adults with no apparent structural heart disease; however, this paradigm has been challenged. It has been postulated that pathological remodeling of the epicardium of the right ventricular outflow tract of patients with BrS leads to decreased cellular excitability and inhomogeneous slowing of cardiac conduction, manifesting as delayed, fragmented electrograms and a type 1 Brugada pattern on ECG: the *depolarization theory*. We have shown that increased fibrosis and dysregulation of gap junctions are present at these sites, and that the application of radiofrequency energy can eliminate the abnormal