Not all Long-QTs Are The Same, Proarrhytmic Quantification with Action Potential Triangulation and Alternans

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Abstract

Long-QT is commonly associated with an increased risk of polymorphic ventricular tachycardia from drug therapy. However, not all drugs prolonging QT interval are proarrhythmic. This study aimed to characterize cellular and tissue mechanisms under which QT-interval prolonging drugs and their combination are proarrhythmic, examining arrhythmia susceptibility due to action potential (AP) triangulation and spatial dispersion of action potential duration (APD). Additionally, we aimed to elucidate that Torsades de Pointe (TdP) associated with long-QT are not necessarily caused by early-after-depolarization (EADs) but are related to the presence of AP alternans in both time and space. Isolated Guinea Pig hearts were Langendorff perfused, and optical mapping was done with a voltage dye-sensitive dye. Two commonly used drugs at the beginning of the COVID-19 pandemic, hydroxychloroquine (HCQ) and Azithromycin (AZM), were added to study the effects of QT interval prolongation. Alternans in time and space were characterized by performing restitution pacing protocols. Comparing APs, HCQ prolongs APD during phase-III repolarization, resulting in a higher triangulation ratio than AZM alone or AZM combined with HCQ. Lower triangulation ratios with AZM are associated with phase-II prolongation, lower arrhythmia, and lower incidence of spatially discordant alternans.

1.   Introduction

QT interval prolongation is commonly associated with proarrhythmic activity, early afterdepolarizations (EADs), and *Torsades de Pointes* (TdP). However, not all drugs prolonging the QT interval are proarrhythmic. To better understand arrhythmia mechanisms, it is necessary to examine how imbalances in ionic membrane currents prolong the action potential duration (APD) and quantify APD spatial repolarization dispersion at the tissue level. EADs occur when action potential (AP) is prolonged in a window from -30 to 0 mV, when previously inactivated L-type Ca channels activate, and I_{CaL} current inflow overcomes the net repolarizing outward K^+ currents. As reactivation of L-type Ca channels takes time, EADs commonly occur at normal to bradycardic sinus rhythms [1,2]. However, as electrotonic coupling between the cells minimizes APD differences among adjacent cells, an EAD in a single cell cannot initiate a propagating AP [3]. Cells across the transmural wall differ in K^+ channels densities resulting in disproportionate APD lengthening with drug-induced blockage of K^+ channels, which in part explains how EADs can act as a focal point or initiate reentry. In clinical practice, transmural APD dispersion is observed as prolongation of ECG's T-wave, and a drug is *torsadogenic* if there is an increase in T-wave duration from its peak till the end [4]. These mechanisms do not fully explain how EADs lead to a generation of propagating AP. Arrhythmia can occur at faster heart rates, at which EADs cannot happen. Spatial dispersion of AP repolarization is a known mechanism leading to reentrant waves resulting from wave blocks and wavebreaks without EADs. In this study, we investigated proarrhythmic markers: APD spatial dispersion and triangulation. Further on, we investigated whether the combination of two drugs prolonging AP under different mechanisms be less proarrhythmic than a drug solely prolonging AP repolarization phase such as class-III antiarrhythmic drugs. We used hydroxychloroquine (HCQ) and azithromycin (AZM) drugs, authorized for emergency use at the beginning of the COVID-19 pandemic. HCQ blocks primarily Ikr and Iks channels leading to a significant prolongation of phase III of AP. On the other hand, Azithromycin moderately blocks potassium channels and prolongs AP in both phases II and III.

2.   Methods

All procedures were approved by the Office of Research and Integrity Assurance at the Georgia Institute

of Technology in accordance with the provisions of the USDA Animal Welfare Act Regulations and Standards. As in the previous studies [5, 6], optical-mapping was used to elucidate the cellular proarrhythmic effects of HCQ and AZM in whole ex vivo arterially perfused GP hearts (n = 12)[7, 8]. Drugs were added to the perfusate circulating through the isolated hearts, at 1-hour mark from the beginning of the experiment (heart cannulation), at concentrations equivalent to therapeutic serum doses of 1000 ng/mL $(3.0\mu M)$ for HCQ and 500 ng/mL $(0.67\mu M)$ for AZM. The hearts were stained with transmembrane potential $(V_m)-$sensitive fluorescent *JPW-6003* dye. $(\pm)$-Blebbistatin at a concentration of 1.8 μM was used as a contraction decoupler. Sequences of fluorescence images were recorded using a high-speed EM-CCD camera (Evolve 128, Photometrics) at the resolution of 128×128 pixels $(0.2 \times 0.2$ mm spatial resolution) at 500 Hz. AP repolarization was quantified as spatial and temporal dispersion of APD as a function of the stimulation period in restitution protocols. Each experiment lasted 5–6 hours, and the time it took for drug effects to reach the plateau in the APD prolongation was around 2–3 hours after administration of the drugs or 3–4 hours from the beginning of the experiment. Restitution protocols were repeated every 30 minutes. As a control, we performed restitution protocols on 2 GP hearts with no drugs and confirmed that AP shape and APD restitution did not change from the beginning of the experiment to the end at the 6-hour mark. Similarly, we performed a restitution protocol before the drugs were added as control at the beginning of all drug experiments. Experiments were done in 14 hearts from 2 years old female Guinea Pigs.

3. Results

The experimental data shows that HCQ, AZM, and HCQ/AZM combination increased APD (Figure 1), the long-QT counterpart at the cellular level. APD prolongation with HCQ was more than the AZM alone and more than the HCQ/AZM combination, indicating that the combination of two drugs is not additive. Histogram plots from APD values across the hear surface show more APD dispersion and prolongation for HCQ than AZM or HCQ/AZM, indicating that HCQ alone is more proarrhythmic with more pronounced period-2 alternans at faster pacing cycle periods (PCL). The triangulation was calculated as APD difference between APD values at 75% repolarization and 30% repolarization divided with the PCL. The triangulation index was calculated for a range of PCL and averaged across imaged heart ventricles.

We calculated the triangulation of APD for the four cases (Normal, HCQ, AZM, and HCQ/AZM). We found that for Normal, AZM and HCQ/AZM triangulation increased with the faster stimulation period. However, for
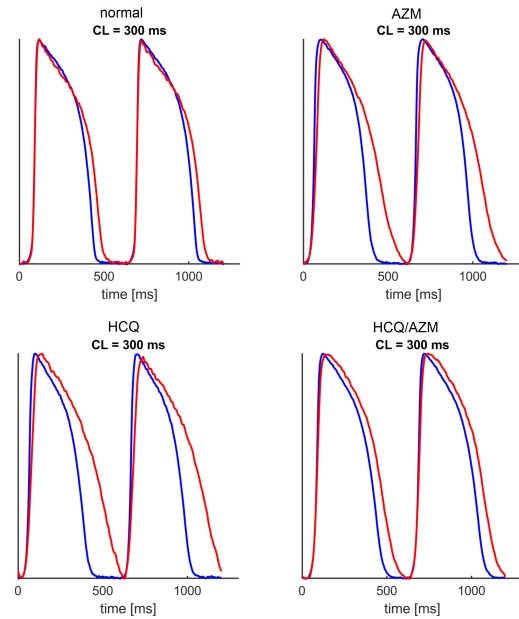

Figure 1. Examples of two AP during constant pacing at 300ms for normal GP, AZM, HCQ and HCQ/AZM. Not only there is an increase in APD (corresponding to long-QT) but also triangulation is more enhanced for HCQ.

HCQ, triangulation was very pronounced and independent of the pacing period, as shown in Figure 3. In addition, there is a strong correlation between the high degree of triangulation with the development of alternans. Our results indicate that high triangulation is well correlated with alternans, that HCQ, compared to the other three cases, has stronger triangulation, larger region of CL for alternans, and easier to induce discordant alternans in space and initiation of fibrillation [7, 8].

4. Discussion

Rate-dependent shortening of APD at shorter CLs is an essential property of cardiac myocytes. It occurs due to incomplete deactivation of IKs currents, serving as a protective antiarrhythmic mechanism to allow long enough diastolic intervals [9]. As HCQ partially inhibits both IKr and IKs, APD prolongs, leading to APD alternans at shorter PCLs than no drug (Figure 1 and 2). Temporal alternans lead to spatial APD alternans. First, at slower CLs, across the entire tissue, APD is prolonged at one beat and shortened in the next beat. Due to dynamical instabilities in wavefront propagation and tissue heterogeneities, concordant alternans transition at shorter CLs to dangerous discordant alternates, which are correspondent to ECG's T-wave alternans, associated clinically with impending ventricular arrhythmias and increased risk of sudden cardiac arrest [10]
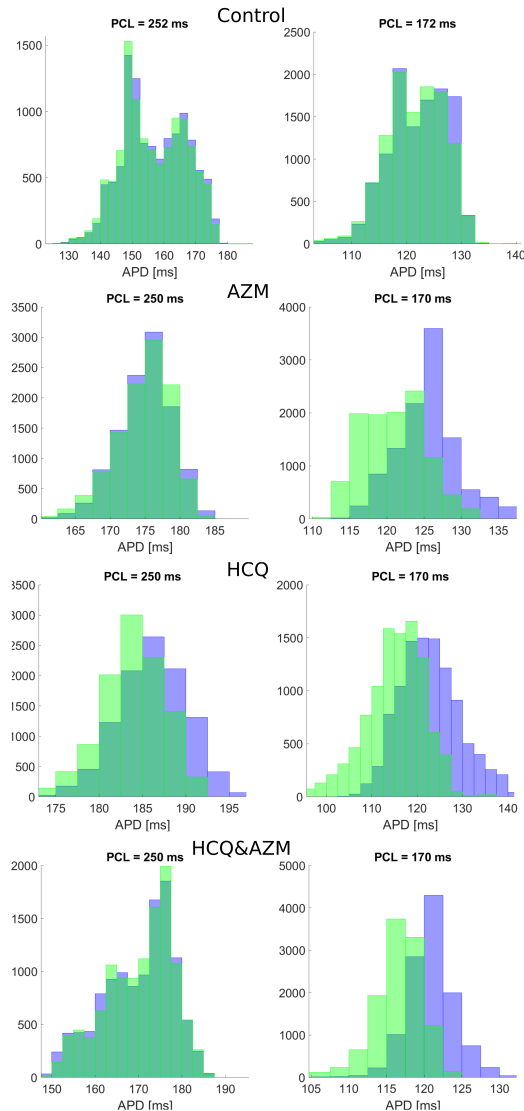
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Figure 2. Histograms of APD for even and odd beats for two different pacing periods (250 and 170ms). Alternans are more pronounced for both periods in HCQ compared to normal, AZM and HCQ/AZM.
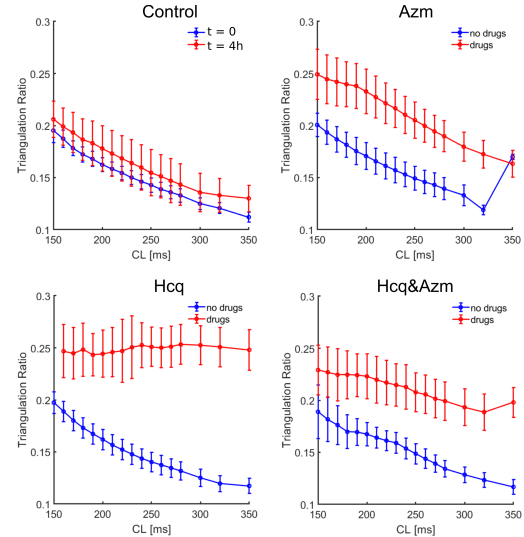

Figure 3. Triangulation of AP as a function of the PCL for Normal, AZM, HCQ and HCQ/AZM for before and after the drugs. There is a clear tendency in triangulation increase with shorter PCL except for HCQ where triangulation is already high and independent of PCL.

Optical mapping experiments on whole hearts due to high spatial and temporal resolution are advantageous to study spatiotemporal dynamics of APD prolongation, compared to single cell or cell culture studies with no cell to cell electrotonic coupling. IKr and IKs channels density varies transmurally, and in our experiments, we used deep red (660 nm) excitation light which has 50% attenuation at tissue depth exceeding 5 mm, allowing measurement of AP not only at high resolution along the ventricular surface but also recorded signals represent integral activity across the ventricular wall.

The extent of QT prolongation has been used as an important marker for arrhythmia. Commonly for all torsadogenic drugs is that they prolong repolarization through inhibition of IKr current (Haverkamp 2000). We aimed to study different electrophysiological effects of AP phase-II and phase-III prolonging drugs and their combination. HCQ increased triangulation, spatial APD dispersion, and APD instability. AZM has, in contrast, a moderate increase in triangulation, spatial APD dispersion, and APD instability index. Despite prolonging the QT interval, AZM case reports have been rare [11] and experiments in isolated rabbit hearts did not report EADs of TdP [12]. Adding AZM, which prolongs AP in a rectangular fashion, to HCQ, which prolongs AP in a triangular pattern, decreases spatial APD dispersion and triangulation. Both drugs have different torsadogenic potential. APD is an important factor in arrhythmogenesis and heterogeneity of APD. While nowadays widely accepted that QT prolongation cannot serve as a single marker for cardiotoxicity in our studies, we did not observe additive effects of HCQ in AZM in AP phase-III prolongation. For us, this represents a new and so far unexplained antiarrhythmic potential with a combination of different QT-prolonging drugs. The prolongation of APD by AZM is different from that of HCQ. With AZM, there was moderate triangulation with little instability. HCQ induced a marked instability, a prominent triangulation. Comparing proarrhythmic of AZM, HCQ, and HCQ/AZM was done by performing a rapid restitution protocol. Long-QT syndrome is characterized by prolonged duration of ventricular repolarization, and it

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is commonly associated with potentially deadly TdP. On the cellular level, the prolonged QT interval is equivalent to prolonged APD as the primary mechanism of class-III antiarrhythmic drugs. The prolongation of the effective refractory period facilitates the termination of reentrant tachyarrhythmias as the wavelength of a propagating wave increases. However, class-III drugs increase the risk for TdP, making the benefit-risk ratio of the drugs questionable [13, 14]. Failure of major clinical trials over the last two decades testing class-III drug candidates can be partially attributed to incomplete human and animal models used in pre-clinical testing, requiring a fundamental understanding of arrhythmia mechanisms across different species [15].

The changes in K^+ channels expression tend to have regional variations in the morphology and duration more pronounced as faster heart rate leading to spatially discordant alternans [5]. Species dependence on IKr and IKs are important in susceptibility to Long-QT-associated arrhythmias. In particular, IKr current is recognized as a critical target for understanding the cardiotoxicity outcomes of many drugs. In the absence of triangulation of the action potential, it may be safe to prolong the QT interval.

Limitations: We did not study the effects of the drugs at slower CLs when EAD induction is possible. We postulate that if any EADs are associated with phase-II prolonging drugs, no EADs, not TdP, will occur when a phase-III drug is combined with a phase-II prolonging drug given at a certain concentration.

Acknowledgments

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