Multi-cardiac ion channel block profiles for lopinavir, ritonavir, chloroquine, and vanoxerine align with clinical ECG changes and proarrhythmia: two case studies in support of the CiPA initiative

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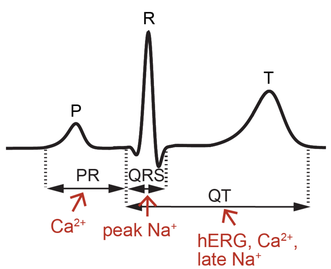
Abstract and Introduction

Background. The Comprehensive in vitro Proarrythmia Assay (CiPA) initiative proposes to use nonclinical data and mechanistic model of the ventricular myocyte to predict Torsade De Pointes (TdP) risk beyond clinical QT_C prolongation. Under the CiPA paradigm, drug effects on cardiac ion channels underlying the ventricular action potential should lead to reasonable predictions of drug-induced electrocardiogram (ECG) changes and TdP risk. However, two clinical studies have reported ECG changes[3] or TdP cases[1] not anticipated based on patch-clamp data of four drugs[4, 5]. In Vicente's study[3], chloroquine and lopinavir/ritonavir are associated with PR and QRS prolongation, suggestive of block of cardiac Ca^{2+} and Na^+ channels that mediate the peak component, respectively. However, Crumb's study[5] showed marginal block of those channels. Obejero-Paz's study[4] reported that vanoxerine blocks hERG and Ca^{2+} channels with similar potencies – a block profile not expected to be associated with TdP risk. However, Piccini's study[1] reported that 3 out of 26 patients developed TdP. *The goal of this study is to assess cardiac ion channel block profiles by these drugs using more physiologically relevant protocols to reexamine alignment of cardiac ion channel data with drug-induced ECG changes.*
Methodology. Effects of chloroquine, lopinavir, ritonavir, and vanoxerine on cardiac hERG, Ca^{2+}, and Na^+ channels were assessed at near physiological temperature using voltage waveforms that mimic ventricular action potentials and following best practices. Block potencies were reported as the concentration associated with 50% inhibition (IC_{50}).
Results. Lopinavir and ritonavir showed similar block of hERG and Ca^{2+} channels at the clinical unbound maximal plasma concentration (free C_{max}). Chloroquine showed preferential block of hERG and some Ca^{2+} and peak Na^+ component at C_{max}. Vanoxerine showed preferential block of hERG channels.
Conclusions. Cardiac ion channel effects of these drugs aligned well with clinical ECG and proarrhythmia findings. This study underscores the importance of assessing drug block of cardiac ion channels using physiologically relevant experimental protocols to enhance translation of nonclinical findings to clinical outcomes.



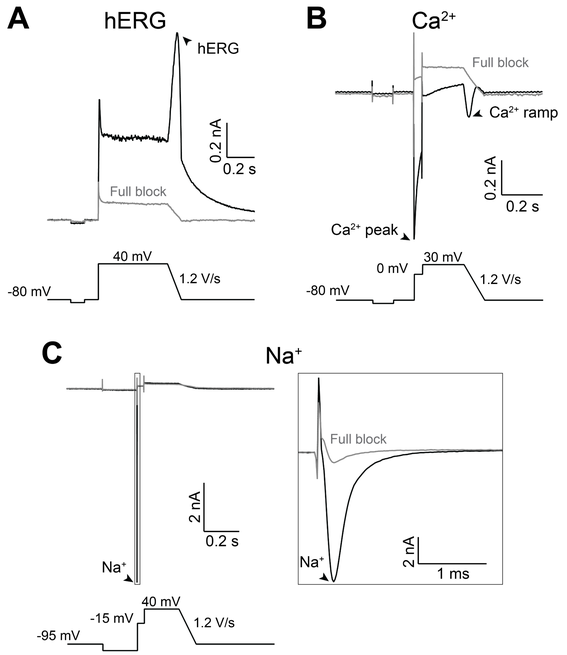
ECG signal reflects activities of ion channels in different parts of the heart. The PR interval is initiated by depolarization of the pacemaker due to activities of Ca^{2+} and other ion channels. Drugs that block Ca^{2+} channels prolong the PR. The QRS interval is started by ventricular depolarization, and is prolonged by drugs that block Na^+ channels that mediate the fast or peak component. During the QT interval, the ventricles depolarize and repolarize. Drugs that impact repolarization cause QT changes: hERG blockers (↑QT), Ca^{2+} blockers (↓QT) and Na^+ channel blockers - late component (↓QT).

Drug	Clinical finding	Previous nonclinical finding
Lopinavir/Ritonavir	PR ↑[3] QRS - QT ↑	***Lopinavir*** Preferential hERG block[5] ***Ritonavir*** Preferential hERG and some Ca^{2+} and late Na^+ block[5]
Chloroquine	PR ↑[3] QRS ↑ QT ↑	Block of hERG[5] No Ca^{2+} or Na^+ block[5]
Vanoxerine	TdP in 3 of 26 patients[1]	Similar hERG and Ca^{2+} block[4]

Materials and Methods

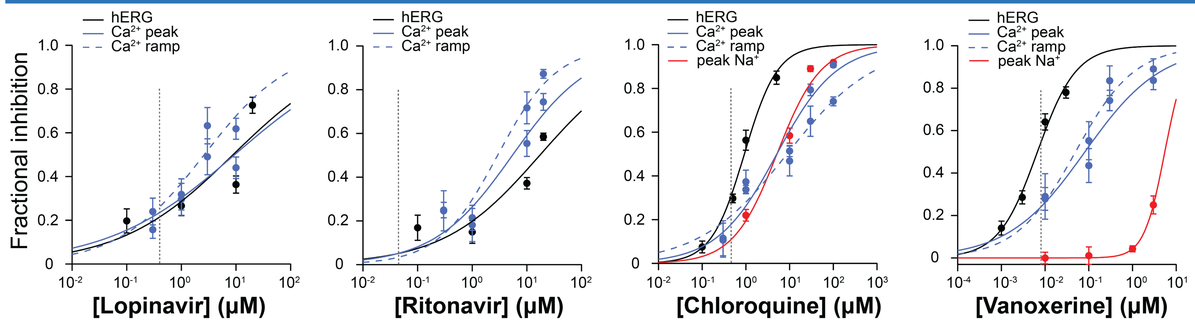
Cell lines. The cells lines used were CHO $hCa_V1.2$ α1C, β_2, $\alpha_2\delta$ for Ca^{2+} channel experiments (CRL), HEK293 $hNa_V1.5$ α β_1 for Na^+ channel experiments (SB Drug Discovery), and HEK293 hERG1a for hERG experiments (Gail Robertson, UW Madison).

Electrophysiology. Cells were recorded at near physiological temperature (37 ± 2°C), using manual whole-cell patch clamp method and protocols found at https://www.fda.gov/media/131157/download.



A. Sample trace and voltage protocol used to measure drug effects on hERG channels. The step-ramp protocol was intended to mimic the voltage changes during a ventricular action potential. hERG current was measured at the peak during the repolarization ramp. **B.** Ca^{2+} channel recordings. Whole cell current was measured during the 0 mV step (Ca^{2+} peak) and during the ramp (Ca^{2+} ramp). **C.** Sample trace and voltage protocol used to elicit Na^+ current. Peak Na^+ current was measured during the -15 mV step. Full block traces (gray) were recorded in the presence of saturating concentrations of respective blockers to eliminate the current under study.

Results and Discussion



Concentration-inhibition plots show the fractional inhibition of each ion channel current by the drugs. Data points presented are mean ± SE of the fractional inhibition values at each concentration (4-5 cells per concentration). To obtain IC_{50} and n_H (Hill slope), individual fractional inhibition values were fit with the Hill equation. Dotted gray lines are the free C_{max} for each drug obtained from Søgaard et al., 1990[2] and Vicente et al., 2019[3].

	Lopinavir				Ritonavir				Chloroquine				Vanoxerine			
	IC_{50}	n_H	% block at C_{max}		IC_{50}	n_H	% block at C_{max}		IC_{50}	n_H	% block at C_{max}		IC_{50}	n_H	% block at C_{max}	
			Nominal	Corrected			Nominal	Corrected*			Nominal	Corrected*			Nominal	Corrected*
hERG	8.9	0.4	21	25	17.2	0.5	5	5	0.9	1.2	22	27	0.007	1.0	55	74
Ca^{2+} peak	10.2	0.4	24	27	5.2	0.6	5	6	4.9	0.7	14	16	0.08	0.5	24	33
Ca^{2+} ramp	2.5	0.6	25	30	2.8	0.8	3	4	8.6	0.4	19	20	0.05	0.6	24	36
Peak Na^+									5.1	0.9	8	9	5.8	1.8	0	0

*Corrected data accounts for drug losses in the recording apparatus due to non-specific binding. Losses were determined in hERG recording solution and were extrapolated for other channels.

Conclusions

At free C_{max},
- Lopinavir and ritonavir both showed similar hERG and Ca^{2+} channels block and increased potency for Ca^{2+} channels at higher concentrations, consistent with the increase in the PR and QT intervals.
- Chloroquine showed hERG, Ca^{2+}, and peak Na^+ channel block. These nonclinical data align with the observed prolongation of the QT, PR, and QRS intervals.
- Vanoxerine preferentially blocks hERG channels over other channels. This block profile is suggestive of TdP risk hence explains TdP cases seen in patients.
- *Assessing drug block of cardiac ion channels using physiologically relevant experimental protocols enhances the translation of nonclinical findings to clinical outcomes.*

This work can be improved by:
1) Further increasing stimulation frequencies to evoke ionic currents within the heart rate range to better estimate % block at C_{max}, since drug block can be frequency-dependent.
2) Verifying concentrations of drugs exposed to all ion channels under study.

References:
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