In vitro potency assessment of hERG inhibitors: cell-based thallium-sensitive fluorescence assay vs. automated electrophysiology

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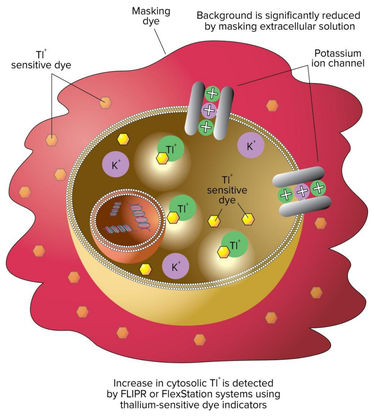
OVERVIEW

The human *ether-á-go-go related* gene (hERG) encodes a voltage-gated potassium channel, which plays a critical role in the repolarization of the cardiac action potential. Disruption of hERG channel activity may cause QT prolongation, resulting in fatal ventricular arrhythmia such as Torsade de Pointes. Pro-arrhythmic activity of FDA approved drugs has resulted in the withdrawal of many drugs from the market. Early detection and elimination of new chemical entities with hERG liability would substantially improve drug safety and minimize the cost of drug development. Conventional whole cell patch clamp (WCPC) recording is considered to be the "gold standard" for studying biophysical properties and pharmacology of ion channels; however, the low throughput nature of the technology has limited its application at early stages of compound triage. Although cell-based fluorescence assays have been developed in the past with reasonable throughput to screen large volume compounds for potential hERG liability, the correlation of compound potency with patch clamp data is relatively low. Furthermore, it could not reliably rank compounds of similar potency. Using a newly developed thallium-sensitive fluorescence dye, we have optimized and validated a robust cell-based hERG fluorescence assay on the FLIPR Tetra® Instrument. The Z' factors ranged from 0.82 to 0.91 in five test occasions. More importantly, potency assessment of known hERG blockers matched those obtained from the automated electrophysiology platform, the IonWorks® Barracuda system, with an identical rank order of potency. In conclusion, the newly validated thallium-sensitive fluorescence dye-based assay performed on the FLIPR Tetra Instrument has the throughput capability for early stage *in vitro* hERG assessment and the accuracy of potency assessment comparable to that obtained by electrophysiological recording techniques.

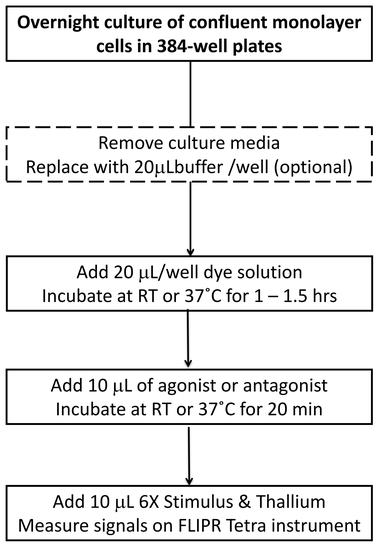
INTRODUCTION

Potassium Assay Principle



- Assay using a novel Tl^+ -sensitive fluorescent dye and a special masking dye
- Fluorescence signals elicited with either a mixture of K^+ and Tl^+ (e.g. voltage-gated channel) or a ligand in the presence of Tl^+ (e.g. ligand-gated channel)
- Functional measurement of channel activities

FLIPR Potassium Assay Kit Work Flow



METHODS

- Chinese hamster ovary (CHO) cells stably transfected with human $K_V11.1$ channel were provided by ChanTest Corporation (Cleveland, OH). All reference hERG inhibitors used in this study were obtained from Sigma-Aldrich (St. Louis, MO)
- Thallium assays were performed using FLIPR Potassium Assay Kit (Molecular Devices) or FluxOR Assay Kit (Life Technologies, Carlsbad, CA) following the manufacturers protocol.
- Fluorescence signals were elicited by 1mM Tl^+ and 10 mM K^+ and detected on FLIPR Tetra instrument using the 470-495 nm LED and the 510-535 nm emission filter. Data were acquired at 1-second interval for 135 seconds.
- Effects of hERG inhibitors were also examined using automated electrophysiology platform, IonWorks Barracuda. Voltage protocol is shown in Figure 2.

ELECTROPHYSIOLOGY PROTOCOL FOR hERG CHANNELS

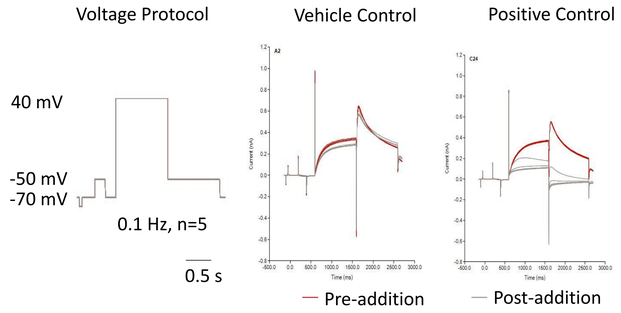
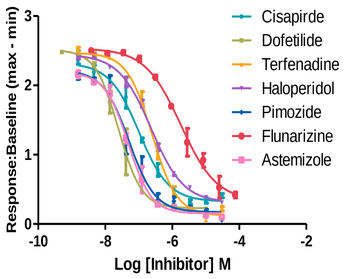


Figure 2. hERG currents were measured using the following voltage protocol: membrane potential was held at -70mV, hyperpolarized to -80mV for to measure membrane resistance, depolarized to -50mV for 200ms to measure background currents; then hERG currents were elicited by depolarization to +40mV for 1s followed by hyperpolarization to -50mV for 1s. The voltage protocol was applied every 10 seconds for five times before and after compound addition. Peak amplitude of hERG tail current at the fifth sweep was used to measure compound effects. Compounds were added at 3X final assay concentration and mixed twice with buffer in the wells to achieve a 1X final compound concentration in 0.3% DMSO. Compounds were incubated for five minutes. Vehicle control: 0.3 % DMSO buffer; Positive control: 4 µM terfenadine.

RESULTS

hERG Channel Pharmacology – IC_{50} Determination of Blockers

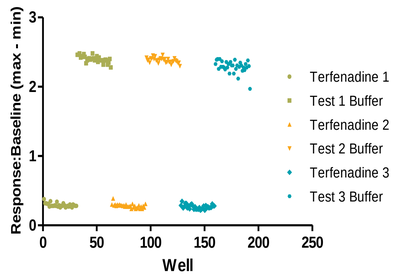
Concentration-Response Curves in FLIPR Potassium Assay



Compound	ClogP	IC_{50} (nM) FLIPR	IC_{50} (nM) IonWorks Barracuda	Fold - FLIPR/IonWorks Barracuda
Dofetilide	1.4	25	15*	1.7
Astemizole	6.1	48	62	0.8
Pimozide	6.4	51	55	0.9
Cisapride	3.7	102	69	1.5
Haloperidol	3.8	237	81	2.9
Terfenadine	6.5	249	332	0.8
Flunarizine	5.4	1795	1,000	1.8

Figure 3. Concentration-response relationship of hERG channel blockers determined using FLIPR Potassium Assay kit and electrophysiology. In the FLIPR assays media was removed to prevent potential serum interference of the pharmacology profile. Dye was loaded at RT for 60 min. Compounds were incubated with the cells for 20 min at RT. Fluorescence signals were elicited by 1mM Tl^+ and 10 mM K^+. Electrophysiology assays were performed using the voltage protocol shown in Figure 2. In both FLIPR and electrophysiology assays, compounds were tested in quadruplicate in 10-point 1:3 gradient. * Value is from published data using IonWorks Quattro.

HTS Assay Robustness-Z' Factor



Test	Z' factor
1	0.89
2	0.91
3	0.86
4	0.82
5	0.88
6	0.85

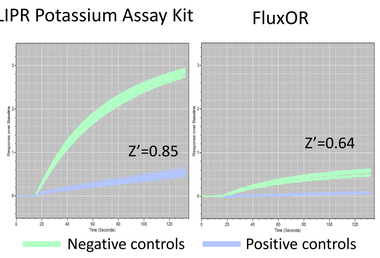
Figure 4. Z' factors were calculated for tests performed on separate days. Day 1: Test 1-3; Day 2: Test 4, Day 3: Test 5 and Day 4: Test 6. Vehicle control: 0.3 % DMSO buffer; Positive control: 4 µM terfenadine. N=32 for Test 1,2,3,5 and 6. N=16 for Test 4.

HTS Assay Robustness-IC_{50} From Separate Experiments

Compound	Test 1 (nM)	Test 2 (nM)	Test 3 (nM)
Dofetilide	25	39	20
Astemizole	48	76	60
Pimozide	51	41	135
Cisapride	102	128	66
Haloperidol	237	241	nd
Terfenadine	249	275	216
Flunarizine	1795	2811	nd

Table 1. IC_{50}s of hERG inhibitors determined in separate tests. N=4.

Kit Comparison



Compound	IC_{50} (nM) FLIPR Potassium Assay	IC_{50} (nM) FluxOR Assay
Dofetalide	39	53
Pimozide	135	195
Terfenadine	275	479

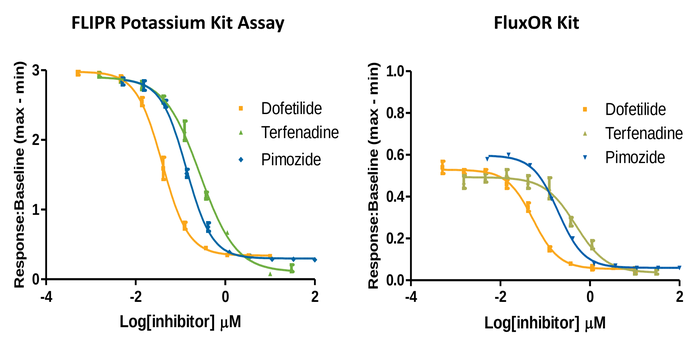


Figure 5. The FLIPR Potassium assay kit shows similar IC_{50} values to the non-homogeneous assay but has significantly higher assay window as indicated by Max values . N=4

CONCLUSIONS

- **The kit shows reduced well-to-well variation, increased signal-noise ratios and improved data quality compared to non-homogeneous formats**
- **The rank order of hERG inhibitors determined in FLIPR potassium kit assay is identical to that in the electrophysiology assay**

MOLECULAR DEVICES