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

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OVERVIEW

The human ether-a-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 Torsades 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

FLIPR Potassium Assay Kit Work Flow

- Overnight culture of confluent monolayer cells in 384-well plates
- Replace with 200µL buffer/well (optional)
- Add 20 µL/well dye solution
- Incubate at RT or 37°C for 1 – 1.5 hrs
- Add 10 µL of agonist or antagonist
- Incubate at RT or 37°C for 20 min
- Add 10 µL 6X Stimulus & Thallium
- Measure signals on FLIPR Tetra instrument

FLIPR Potassium Assay Kit Work Flow

- Remove culture media
- Add 20 µL/well dye solution
- Incubate at RT or 37°C for 1 – 1.5 hrs
- Add 10 µL of agonist or antagonist
- Incubate at RT or 37°C for 20 min
- Add 10 µL 6X Stimulus & Thallium
- Measure signals on FLIPR Tetra instrument

RESULTS

hERG Channel Pharmacology – IC50 Determination of Blockers

Concentration-Response Curves in FLIPR Potassium Assay

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 (nM) FLIPR</th>
<th>IC50 (nM) IonWorks Barracuda</th>
<th>Fold – FLIPR/IonWorks Barracuda</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dofetilide</td>
<td>1.4</td>
<td>25</td>
<td>15*</td>
</tr>
<tr>
<td>Astemizole</td>
<td>6.1</td>
<td>48</td>
<td>62</td>
</tr>
<tr>
<td>Pimozide</td>
<td>6.4</td>
<td>51</td>
<td>55</td>
</tr>
<tr>
<td>Cisapride</td>
<td>3.7</td>
<td>102</td>
<td>69</td>
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<tr>
<td>Haloperidol</td>
<td>3.8</td>
<td>237</td>
<td>81</td>
</tr>
<tr>
<td>Terfenadine</td>
<td>6.5</td>
<td>249</td>
<td>332</td>
</tr>
<tr>
<td>Flunarizine</td>
<td>5.4</td>
<td>1795</td>
<td>1,000</td>
</tr>
</tbody>
</table>

Table 1. IC50 of hERG inhibitors determined in separate tests. 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