CHO-hERG Cell Line

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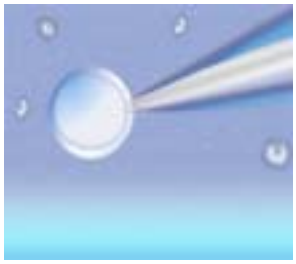
Drug-induced blockage of the *human ether-a-go-go* (hERG) potassium channel is associated with prolonged QT, which can predispose individuals to arrhythmias such as Torsades de Pointes. Therefore, early screening of compounds for hERG channel activity is a vital step in the drug-discovery process. Our CHO-hERG cell line was developed and validated as a tool for screening compounds which may lead to QT prolongation.

Our CHO-hERG cell line:

- **Stably expresses hERG potassium channels**
- **Validated using electrophysiology and flux assay**
- **Suitable for high-throughput screening (HTS)**

Electrophysiology



Electrophysiology experiments were conducted using standard patch clamp techniques.

Validation of CHO-hERG cell line was carried with whole cell patch clamping to show faimily of tail currents, I-V relationships, and IC50 determinations of two positive blockers (Figures 1-4).

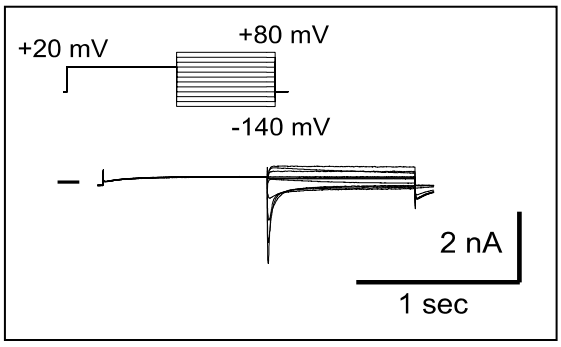


Fig. 1. Family of tail currents from whole-cell patch clamping of CHO-hERG cells

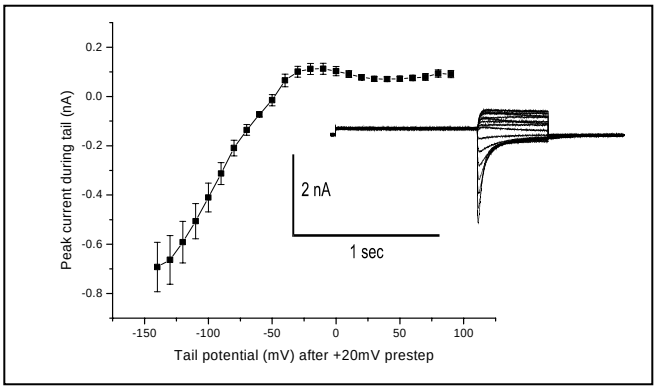


Fig. 2. Current-voltage relationship of membrane current after a depolarizing voltage step to +20 mV.

For IC_{50} experiments, a test pulse was applied until the peak tail current stabilized (Fig. 3 & 4).

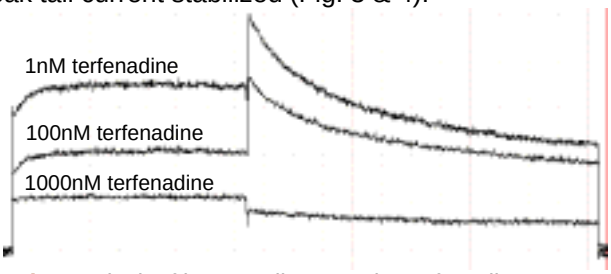


Fig. 3. Block of hERG tail current by terfenadine

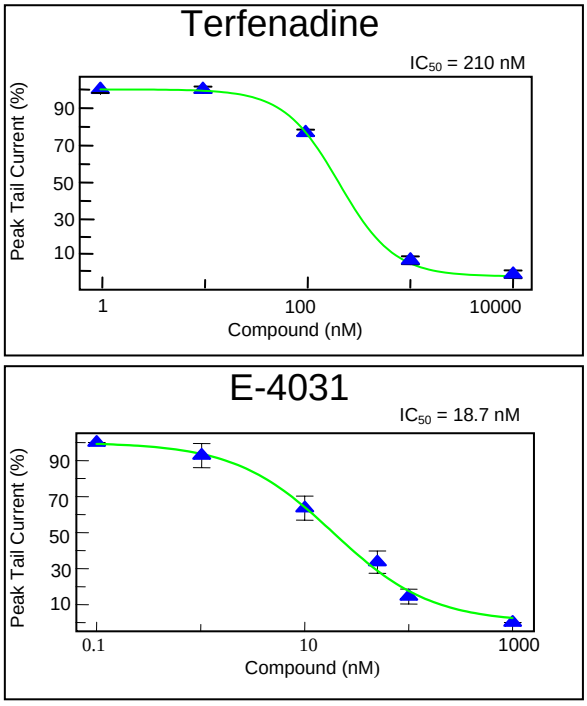


Fig. 4. IC_{50} plots of terfenadine and E-4031 using the CHO-hERG cell line as determined by patch clamp.

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Rubidium Efflux Assay

The CHO-hERG cell line was also validated for cell based flux assay.

Using Aurora Biomed's Rubidium Efflux Assay protocol, basal efflux was measured at 11%. After a 6 minute activation time using 60mM KCl, the maximal activation-induced efflux was 81% (Fig. 5 & 6).

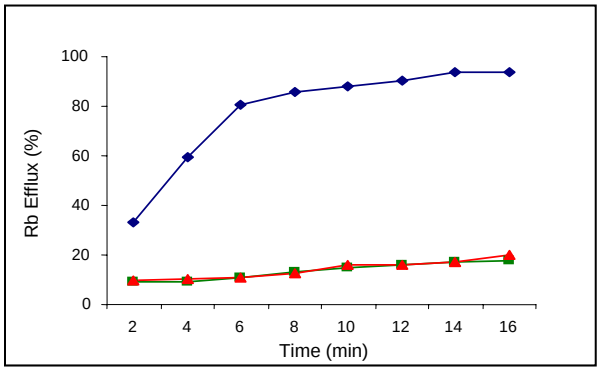

Fig. 5. Activation of hERG channel for different time lengths resulted in an increase of Rb^+ efflux (blue line) whereas basal and blocked efflux with 3 µM astemizole ranged between 8-17% (green line) and 23-76% (red line), respectively.

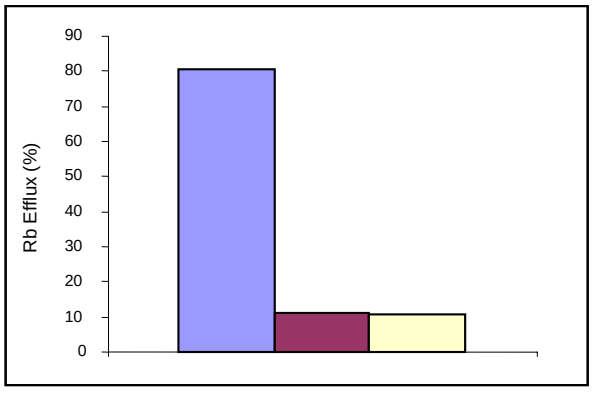

Fig. 6. Activation of hERG in CHO cell line. Activation time of 6 min with 60 mM KCl leads to an 81% activation efflux (blue bar), 11% basal efflux (red bar) and block of 71% flux with 3 uM astemizole (yellow bar).

The concentration-response curves of two commonly known hERG blockers, terfenadine and E-4031, were determined using the CHO-hERG cell line (Fig. 7). Table 1 shows the relative potency of several other hERG blockers as determined using the CHO-hERG cell line and the Rubidium Efflux Assay

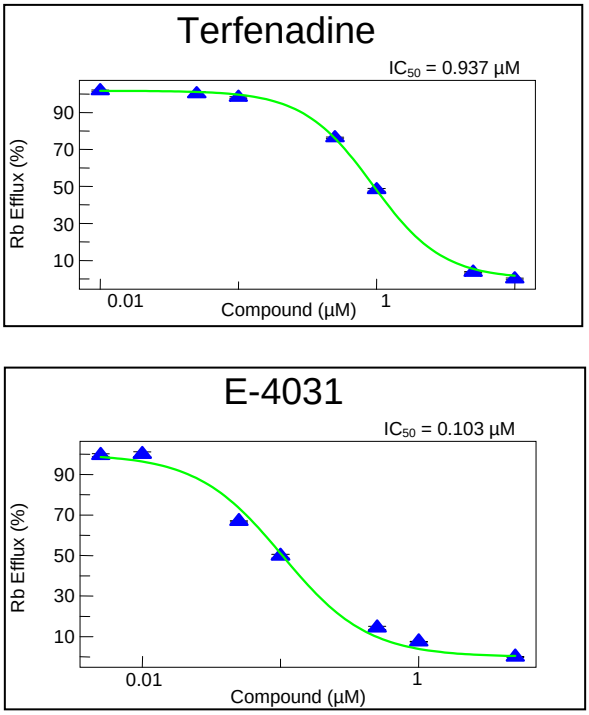

Fig. 7. IC_{50} of terfenadine and E-4031 using the CHO-hERG cell line as determined by the Rubidium Efflux Assay.

Table 1. Relative potencies of hERG blockers as determined by the Rubidium Flux Assay.

Drug	IC_{50} (µM)
Pimozide	0.018
Dofetilide	0.021
Astemizole	0.044
E-4031	0.1
Terfenadine	0.94
Domperidone	2.8
Verapamil	3.5
Thioridazine	3.7
Fluoxetine	4.1
Risperidone	8.6
Diltiazem	62
Disopyramide	120
Aspirin	No block
Chloramphenicol	No block