HEK-hERG Cell Line

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Drug-induced blockage of the *human ether-a-go-go* (hERG) potassium channel is associated with a prolonged QT interval, which may 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 HEK-hERG cell line was developed and validated for use as a screening tool for compounds which may lead to QT prolongation.

Our HEK-hERG cell line:

- **Stably expresses the hERG potassium channel**
- **Has been validated using electrophysiology and flux assay**
- **Is suitable for high-throughput screening (HTS)**

Electrophysiology

Electrophysiology experiments were conducted using standard patch clamp techniques.

Validation of HEK-hERG cell line was carried out using whole cell patch clamping to show the family of tail currents and I-V relationships (Figures 1-2).

As shown in Figure 3, the background HEK conductance is usually less than 1-200pA, maximal and there is no tail current. Under certain culture conditions one sees a shift in the voltage-dependence of activation of hERG such that significant current will be triggered with a prolonged depolarization at even -60mV.

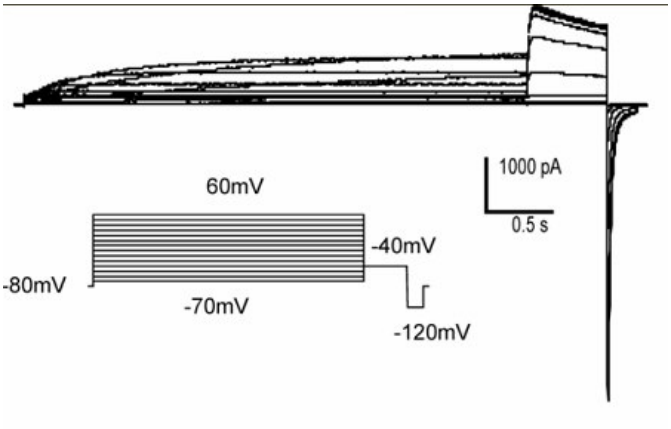

Fig. 1. HERG K+ channel activity transfected in HEK cells. Representative current tracings showing currents were elicited by depolarizing steps to various levels (between 40 and –80mV) followed by repolarizing steps to –70 mV.

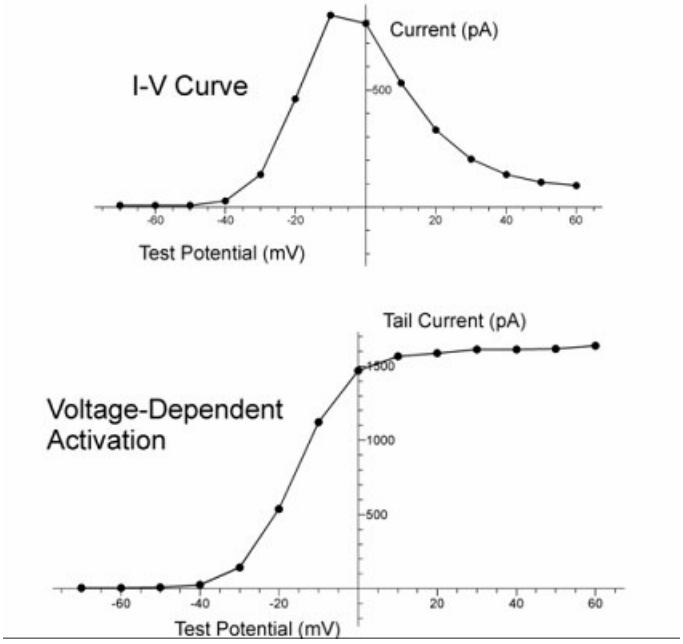

Fig. 2. hERG activity transfected in HEK cells showing I-V relationship, and voltage dependent activation. The voltage dependent activation curves plotted from peak tail currents during a repolarizing step to –70 mV after depolarizing to various voltages. Data are means and S.E., n = 8 per group.

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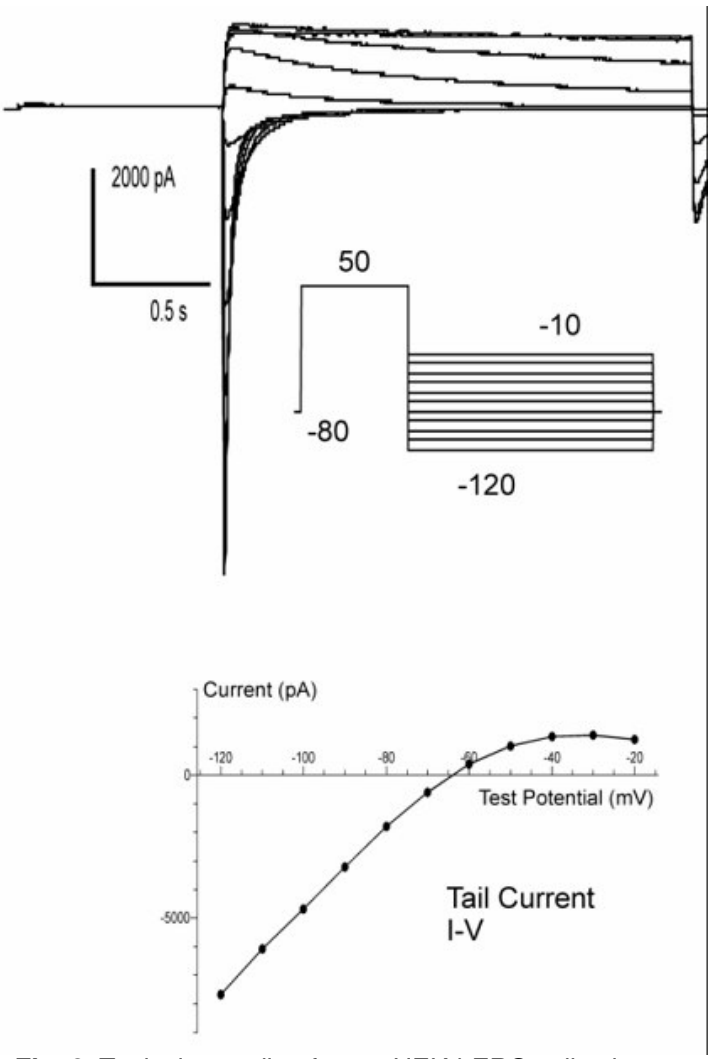


Fig. 3. Typical recording from a HEK-hERG cell using voltage-clamp protocols.

Rubidium Efflux Assay

The HEK-hERG cell line also validated for use with a cell based flux assay.

Using Aurora Biomed's Rubidium Efflux Assay protocol, the basal efflux was measured to be 5%. Following a 6 minute activation time using 60mM KCl, the maximal activation-induced efflux was 87, providing a more than 5-fold window of detection (Figure 4).

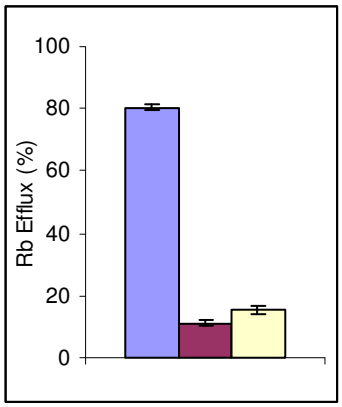
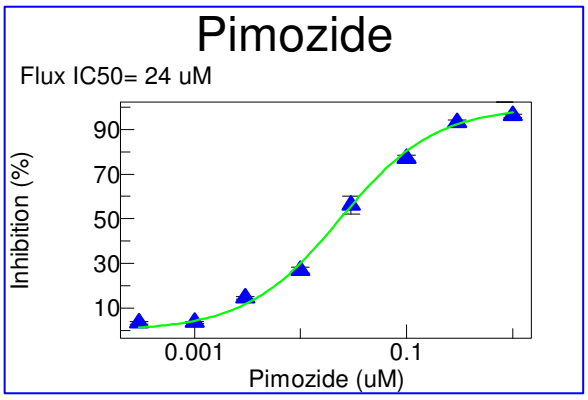


Fig. 4. Activation of hERG in the HEK cell line. An activation time of 6 minutes with 60 mM KCl leads to an 86% activation efflux (red bar), a 15% basal efflux (blue bar), and inhibition by 1 µM astemizole (yellow bar).

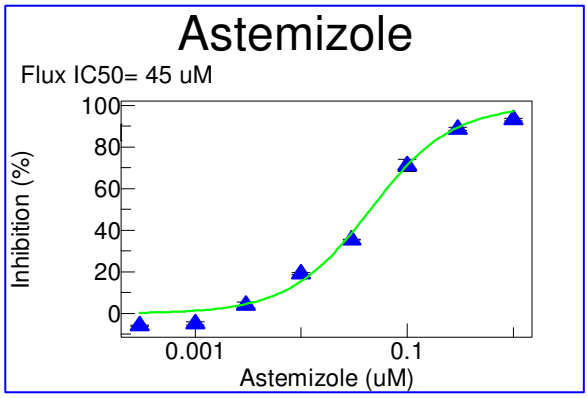
The concentration-response curves of three common hERG blockers, pimozide, astemizole and terfenadine, were determined using the HEK-hERG cell line. Figure 5 shows the IC_{50} determinations of these three positive blockers. Table 1 shows the relative potency of these hERG blockers as determined using the HEK-hERG cell line and the Rubidium Efflux Assay.

Lastly, we calculated the concentration-response curves of normalized Rb+ flux data for these three commonly known hERG blockers, pimozide, astemizole and terfenadine, using a maximum concentration of 1 µM, 1µM, and 3 µM respectively, as shown in Figure 6.

a)



b)



c)

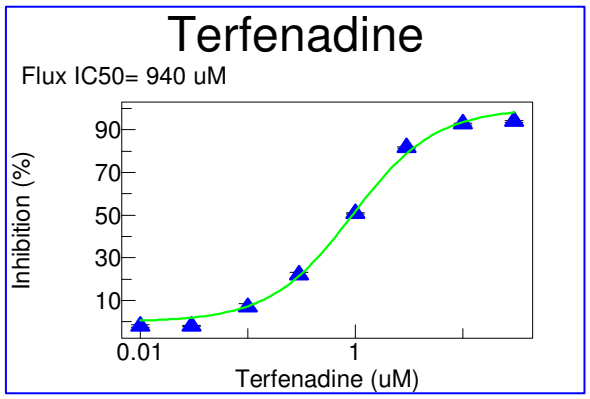


Fig. 5. IC_{50} of (a) pimozide, (b) astemizole, and (c) terfenadine on HEK-hERG cell line as determined by the Rubidium Efflux Assay.

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

Drug	IC_{50} (µM)
Pimozide	0.024
Astemizole	0.045
Terfenadine	0.940

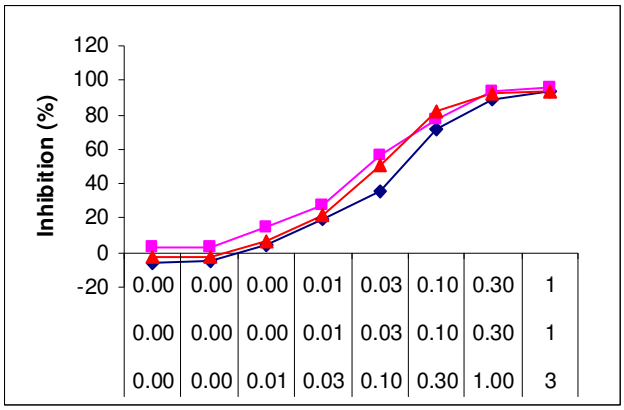


Fig. 6. The concentration-response curves of normalized Rb^+ flux data of pimozide, astemizole, and terfenadine.