

hERG Binding Assays for the Identification of Potentially Cardiotoxic Compounds



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1 Introduction

Several classes of drugs produce life-threatening cardiac toxicity associated with the prolongation of the long QT interval. This toxicity is caused by hERG K⁺ channel blockade and can result in the costly withdrawal of drugs or late stage termination of drug development. The early detection of potential cardiotoxicity can yield significant cost and time savings. A high-throughput binding assay can rapidly and cost-effectively identify compounds that interact with the hERG channel.

We have developed a stable hERG-expressing line in HEK-293 cells. Electrophysiological data show peak and tail currents for individual cells of about 500 pA. Radioligand binding studies using tritiated astemizole and iodinated BeKm-1 demonstrate a good expression level (B_{max} value) and a pharmacological profile that is in agreement with published data. The complete pharmacological characterization of the hERG K⁺ channel in crude membrane preparations will be presented and will include data generated using various binding assay platforms.

2 Methods

Cell Line and Crude Membranes: A stable cell line expressing hERG K⁺ channels was generated by transfecting the hERG1 cDNA into HEK-293 cells. Crude membranes were prepared as described by Angelo *et al.* (6) and stored at -80°C.

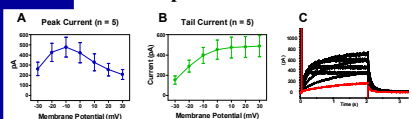
[³H]-Astemizole Binding Assays: [³H]-astemizole was obtained from PerkinElmer (NET1140). Assays were performed in 200 µL of 50 mM Hepes pH 7.4, 60 mM KCl, 0.1% BSA with 2.5 µg of membranes.

[¹²⁵I]-BeKm-1 Binding Assays: [¹²⁵I]-BeKm-1 was obtained from PerkinElmer (NEX412). Assays were performed in 200 µL of 20 mM Hepes/Tris pH 7.2, 100 µM KCl, 0.1% BSA with 2.4 µg of membranes.

Filtration Assay Procedure: Assay mixtures were incubated for 1 h at room temperature (RT) and filtered through Filtermat-A pre-soaked in 0.3% PEI. Signal was detected with a MicroBeta® (PerkinElmer LAS Inc.).

Homogeneous FlashBlue™ [¹²⁵I]-BeKm-1 Binding Assays: Assays were performed in Optiplates-384 in 40 µL of the BeKm-1 assay buffer with 0.5% BSA using 62.5 µg of FlashBlue GPCR beads and 1.25 µg of membranes per well. Plates were spun for 5 min at 1000 RPM after a 1 h incubation at RT prior to signal detection with a TopCount® (PerkinElmer LAS Inc.).

3 Electrophysiological Properties of hERG Expressed in HEK-293 cells



Panels A and B show step and tail currents from hERG expressed stably in HEK-293 cells after 40 passages. Whole cell patch clamp was used to record currents at 37°C. The holding potential was -80 mV and 2 second depolarizing pulses were applied up to +30 mV. Tails were recorded at -50 mV. Data are presented as mean ± SEM. Panel C shows a family of hERG currents from an experiment described above. hERG currents in our cell line are typical and consistent with expression in other hERG transfected cell lines.

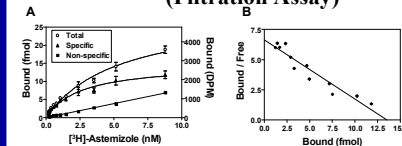
4 Results

Astemizole binds preferentially to the open and/or inactivated states of hERG (1, 2). Saturation binding with [³H]-astemizole (panel 5) indicates a B_{max} value for our hERG cell line of 6 pmol/mg and a K_d value for the radioligand of 3 nM. K_d values obtained for various hERG blockers for [³H]-astemizole binding (panel 6) are in accordance with published values (3, 4).

The scorpion toxin BeKm-1 (5, 6) blocks hERG preferentially through closed channels (2, 7). Both filtration and homogeneous FlashBlue hERG binding assays have been optimized with [¹²⁵I]-BeKm-1 (Panels 7-11). Filtration binding assays indicated that our hERG K⁺ channel membrane preparations a B_{max} value for [¹²⁵I]-BeKm-1 of 0.5 pmol/mg, with a K_d value of 0.13 nM (panel 7). Competition of [¹²⁵I]-BeKm-1 binding by known blockers indicated K_i values comparable to published values (3, 4), except for astemizole. K_d and K_i values obtained with homogeneous FlashBlue assays were similar (panel 10 and data not shown).

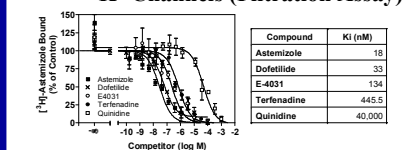
Notably, the B_{max} value for [³H]-astemizole binding in hERG K⁺ channel membrane preparations is about 10-fold higher than that for [¹²⁵I]-BeKm-1. This might reflect the preferential binding of the two radioligands to different conformational states of the hERG K⁺ channels.

5 Saturation Binding of hERG K⁺ Channels with [³H]-Astemizole (Filtration Assay)



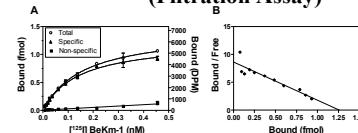
[³H]-Astemizole saturation binding assay performed on hERG K⁺ channel membranes. B_{max} value of 6 pmol/mg and K_d value of 3 nM were obtained. Panel A illustrates a typical saturation binding curve. The corresponding Scatchard analysis is illustrated in panel B. The data presented is derived from single site fitting.

6 Competition for [³H]-Astemizole Binding to hERG K⁺ Channels (Filtration Assay)



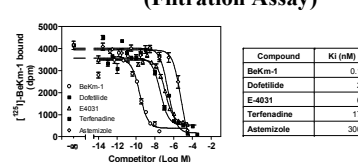
Competition of known hERG blockers for the binding of [³H]-astemizole to hERG K⁺ channels. The radioligand was used at a concentration of 4.4 nM. K_i values are the average of two independent experiments.

7 Saturation Binding of hERG K⁺ Channels with [¹²⁵I]-BeKm-1 (Filtration Assay)



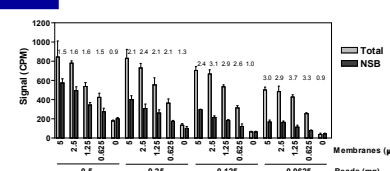
[¹²⁵I]-BeKm-1 saturation binding assay performed on hERG K⁺ channel membranes. A B_{max} value of 0.5 pmol/mg and K_d value of 0.13 nM were obtained. Panel A illustrates the saturation binding curve. The corresponding Scatchard analysis is illustrated in panel B. The data presented is derived from single site fitting.

8 Competition for [¹²⁵I]-BeKm-1 Binding to hERG K⁺ Channels (Filtration Assay)



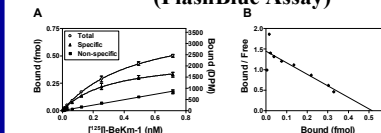
Competition of known hERG blockers for the binding of [¹²⁵I]-BeKm-1 to hERG K⁺ channels. The radioligand was used a concentration of 0.17 nM. K_i values are the average of two independent experiments.

9 Homogeneous FlashBlue Binding Assay with [¹²⁵I]-BeKm-1



A homogeneous hERG binding assay was developed using FlashBlue GPCR beads and [¹²⁵I]-BeKm-1 (0.1 nM) in 384-well format. Best assay conditions were 0.0625 mg beads and 1.25 µg membranes, with a signal to background (S/B) ratio of 3.7.

10 Saturation Binding of hERG K⁺ Channels with [¹²⁵I]-BeKm-1 (FlashBlue Assay)



[¹²⁵I]-BeKm-1 saturation binding assay was performed on hERG K⁺ channel membranes using FlashBlue beads. An average B_{max} value of 0.6 pmol/mg (considering a counting efficiency of 40% for FlashBlue beads) was obtained, with a K_d value of 0.17 nM. Panel A shows a saturation binding curve. The corresponding Scatchard analysis is illustrated in panel B. The data presented is derived from single site fitting.

11 Conclusions

- We have developed a stable hERG/HEK-293 cell line with high-level expression for hERG showing typical hERG currents.
- hERG K⁺ channel membranes have a B_{max} of about 6 pmol/mg of proteins for [³H]-astemizole and of about 0.5 pmol/mg of proteins for [¹²⁵I]-BeKm-1.
- K_d values and order of potency of known hERG blockers are in agreement with published data (3, 4), except for astemizole that displaces only weakly [¹²⁵I]-BeKm-1, possibly due to its poor interaction with closed channels, or to its interaction with a different binding site.
- Availability of two radioligands with distinct conformational specificity allows identifying blocking compounds interacting with various states of the hERG channel

12 References

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