Assessment Of hERG Blockade By Radioligand Binding Assay With hERG Membrane Preparations

Mahmoud Zubaidi[1], Mark Santos[1], Joelle Wolfson[1], Zaheda Farzin[1], Umesh Patel[3], Duncan Jarman[3], Jeff Clare[3], Blaine Armbruster[2], Jeff Till[2], Luke Armstrong[1], Matthew Hsu[1]*

Millipore Corporation, [1]Temecula CA, USA, [2]St. Charles MO, USA, [3]Cambridge, UK; *Corresponding Author: Matthew_Hsu@millipore.com

Abstract

The human ether-a-go-go related gene (hERG) is a potassium ion channel which is essential for normal cardiac repolarization. In drug screening models, the hERG K+ channel is inhibited by a wide variety of compounds, and its blockage can lead to cardiac QT interval prolongation and life threatening arrhythmias. Cardiac safety relating to I_{Kr} K+ channels has become a major concern of regulatory agencies, as hERG channel inhibition has been identified as the firmest link to QT prolongation.

In order to identify potential cardiac liabilities for compounds at an early stage in drug discovery process, fast and cost effective in vitro assays for hERG channel inhibition are desirable. We have developed radioligand binding assays using a membrane preparation from a high-level hERG-expressing HEK293 cell line, which has previously been well-characterized by electrophysiology. The membranes were characterized by radioligand binding assays with three different radioligands, [^{3}H]-astemizole, [^{3}H]-dofetilide and [^{125}I]-BeKm to assess the pharmacology; and the rank order of 8 various small molecule hERG inhibitors using [^{3}H]-astemizole binding is consistent with the pharmacological values obtained from live cell-based assays using electrophysiology. In summary, the hERG membrane preparations have Bmax values greater than 10pmol/mg using [^{3}H]-astemizole binding, and the radioligand assays provide higher throughput with comparable results to the electrophysiology assays using hERG cells.

Introduction

The cardiac action potential mediating the excitation and relaxation of cardiac muscle is choreographed by multiple ion channels. Alteration of function of a number of these ion channels, either by genetic variation or by interaction with administered drugs, can lead to prolongation of the QT interval on the electrocardiogram and the life-threatening arrhythmia Torsades de Pointes (TdP).

The rapid delayed rectifying potassium channel human ether-a-go-go related gene (hERG) has been shown to be the target for the majority of drugs withdrawn from the market due to QT prolongation. Thus, evaluation of candidate compounds for hERG blockade activity by in vitro assay is a critical step in assessing the potential for cardiac liability.

Millipore provides a portfolio of solutions for evaluation of cardiac risk of candidate compounds:

- Cardiac*Profiler*™ Service is a comprehensive panel of assays for cardiac liability testing on candidate compounds.
- For specific evaluation of hERG blocking activity of compounds, Millipore currently offers a panel of PrecisION® hERG cell lines developed in both HEK293 and CHO backgrounds (catalog # CYL 3006 and CYL3039 for hERG in HEK293 background, and CYL3038 for hERG in CHO background).
- In addition, Millipore provides the hERG*Profiler*™ service for analysis of customer compounds with the PrecisION hERG cell lines, by validated methods including manual patch clamp, IonWorks® or PatchXpress® assays.

PrecisION hERG Membrane Preparation

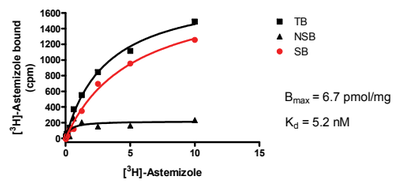
Millipore is pleased to introduce the PrecisION hERG Membrane Preparation (catalog # CYL4039) as another tool for evaluation of compound cardiac liability.

The hERG Membrane Preparation is prepared from the PrecisION hERG-expressing HEK293 cell line (catalog # CYL3039) employed in the hERG*Profiler* service, and we demonstrate here that the pharmacology obtained by radioligand binding with PrecisION hERG Membrane Preparation is comparable to that obtained by electrophysiological methods.

Membranes were prepared from hERG-expressing HEK293 cells (Millipore catalog # CYL3039) by nitrogen cavitation and high-speed centrifugation.

hERG Membrane Preparation exhibits high binding capacity with multiple radioligands

Highest binding capacity is obtained with [^{3}H]-astemizole



Lower binding capacity, but good signal window, is obtained with [^{125}I]-BeKm-1 and [^{3}H]-dofetilide

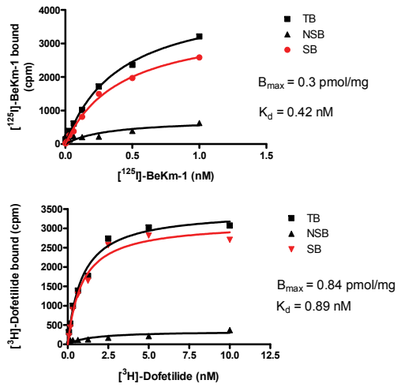


Figure 1.

(A) Binding assay with [^{3}H]-astemizole: 1 unit (10 µg) per well hERG Membrane Preparation is mixed with [^{3}H]-Astemizole (Perkin Elmer) in the absence (total binding, TB) or in the presence (nonspecific binding, NSB) of 500-fold excess unlabeled astemizole. After incubation for 1-2 h, the binding reaction is transferred to a PEI-precoated FB harvest plate (Millipore cat. # MAHF B1H), then washed. The plate is dried and counted. Specific binding (SB) was determined by subtracting NSB from TB.

(B) Binding assay with [^{125}I]-BeKm-1: Performed as above, except 0.5 units (5 µg) per well hERG membrane preparation is incubated with [^{125}I]-BeKm-1 (Perkin Elmer) in the absence (TB) or presence (NSB) of 500-fold excess unlabeled BeKm-1.

(C) Binding assay with [^{3}H]-dofetilide: Performed as above, except 5 units (50 µg) per well hERG membrane preparation is incubated with [^{3}H]-dofetilide (American Radiolabeled Chemicals) in the absence (TB) or presence (NSB) of 1000-fold excess unlabeled dofetilide.

Preserved rank ordering of hERG inhibitors by electrophysiology and binding with [^{3}H]-astemizole and [^{3}H]-dofetilide

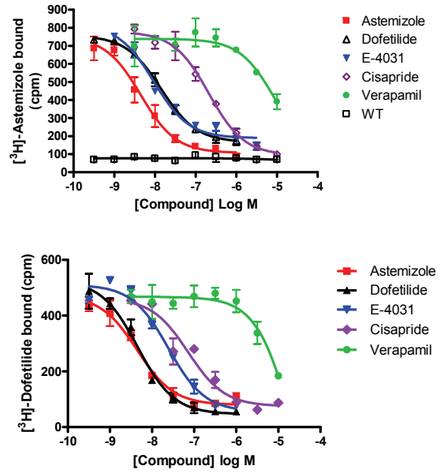


Figure 2.

(A) 10 µg/well hERG membranes were incubated with 3.0 nM [^{3}H]-Astemizole and increasing concentrations of unlabeled compounds in a 96-well plate. (B) 20 µg/well hERG membranes were incubated with 2 nM [^{3}H]-Dofetilide and increasing concentrations of unlabeled compounds in a 96-well plate.

Table 2. Rank order comparison of various small molecule hERG inhibitors using [^{3}H]-astemizole binding assay and E-Phys assays:

	Binding Ki (nM) with hERG membrane preps and [^{3}H]-astemizole		Electrophysiology IC50 (nM) with hERG cell lines		
	Millipore CYL4039 value	Literature value*	Millipore CYL4039 by IonWorks PPC	Millipore CYL4039 by PatchExpress	Literature* value by Patch Clamp
Astemizole	3.4 ± 0.7	3.3 ± 0.7	4.6 ± 0.003	ND	0.5
Dofetilide	5.8 ± 2.4	28 ± 6	ND	ND	15.3 ± 2.5
E-4031	6.1 ± 0.8	58 ± 5	115 ± 0.02	144 ± 44	14
Pimozide	18.8 ± 2.3	14 ± 4	ND	ND	18
Cisapride	104.9 ± 30.9	123 ± 25	75.6 ± 0.02	5 ± 1	44.5 ± 10.6
Haloperidol	241.4 ± 21.4	234 ± 20	ND	ND	63
Risperidone	> 4000	4302 ± 319	ND	ND	167
Verapimil	> 4000	3902 ± 529	1332 ± 120	3100 ± 400	830

* Data obtained from Chiu *et al.* (2004) *J. Pharmacol. Sci.* 95: 311-319

Binding assays indicate that small molecule hERG inhibitors bind to different site than the peptide toxin [^{125}I]-BeKm-1

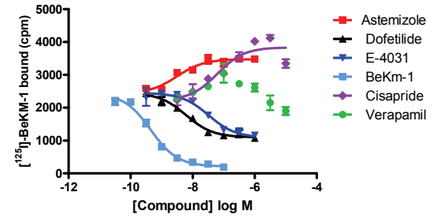


Figure 3.

10 µg/well hERG membranes were incubated with 0.1 nM [^{125}I]-BeKm-1 and increasing concentrations of unlabeled compounds in a 96-well plate. Several modes of activity were observed:

Unlabeled BeKm-1 completely competes with labeled ligand with high potency.

E-4031 and dofetilide partially compete with the labeled BeKm-1 with lesser potency.

Astemizole and cisapride display slight potentiation of labeled BeKm-binding

Conclusions

Millipore's hERG membrane preparations:

- Exhibit high binding capacity and affinity as determined by [^{3}H]-astemizole binding, with a Bmax values of 6-8 pmol/mg.
- Also bind to [^{3}H]dofetilide and [^{125}I]-BeKm-1 with high affinity, but lower capacity, consistent with activation-state dependent binding by these ligands.

Rank ordering of compounds by various methodologies reveal:

- Binding of [^{3}H]-astemizole to Millipore's hERG membrane preparations yields Ki values that closely correlate with IC50 values obtained by electrophysiological measurements performed on PrecisION hERG cell lines.
- Comparison of compounds with three radioligands on Millipore's hERG membrane preparations indicate that compound potencies closely correlate in assays with [^{3}H]-dofetilide and [^{3}H]-astemizole.
- However, binding assays performed with [^{125}I]-BeKm-1 yield different results, with hERG inhibitors displaying either low potency partial inhibition, or potentiation of radioligand binding. This result is consistent with models for separate but overlapping binding sites for peptide toxins and small molecule hERG inhibitors.

Summary

Radioligand binding assays performed with Millipore's PrecisION hERG membrane preparations provide:

- A rapid, high-throughput method for analysis of hERG blocking activity
- Better characterized pharmacologically using multiple radioligands
- Binding results correlate well with those obtained by functional electrophysiological methods
- High signal: background and high affinity binding

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