B'SYS GmbH

hERG Trafficking Assay -Luminescence

Specification Sheet

1. BACKGROUND

1.1. Drug-induced QT Prolongation

The QT interval as measured in the electrocardiogram (ECG) is determined by the duration of the cardiac action potential. The rapid delayed rectifier current (I_{Kr}) is important for cardiac action potential repolarization. Suppression of I_{Kr} function by adverse drug effects (e.g. terfenadine, cisapride, astemizole etc.) can induce a prolongation of the QT interval carrying elevated risk of life-threatening cardiac arrhythmias.

1.2. Regulatory Issues

I_{Kr} is mediated by the potassium channel hERG (human ether-à-go-go related gene). This ion channel is involved in both, congenital and acquired long QT syndrome. According to the ICH S7A and S7B guidelines the analysis of molecules for inhibition of hERG currents allows the assessment of the potential for QT prolongation.

1.3. Indirect reduction of the hERG current by decreased surface expression

Drug-induced, acquired LQTS is often caused by direct blockade of the cardiac potassium channel hERG by a wide variety of structurally diverse therapeutic compounds. It was shown that As_2O_3 produced its effects not by direct block of cardiac ion channels, but indirectly, by inhibiting the processing of hERG protein in the endoplasmic reticulum and reducing the surface expression of this potassium channel (Ficker et al 2004). These effects can not be analyzed by acute electrophysiological experiments but by analysis of the hERG plasma membrane expression.

2. MATERIEL AND METHODS

2.1. Test system

B'SYS' HEK293 hERG HA tag cell line stably expresses a hERG channel with a hemagglutinin (HA) tag in its extracellular loop spanning hERG transmembrane domains S1 and S2. The cell line was validated biophysically using manual patch-clamping. No significant changes compared to WT hERG channels were identified.

2.2. Method

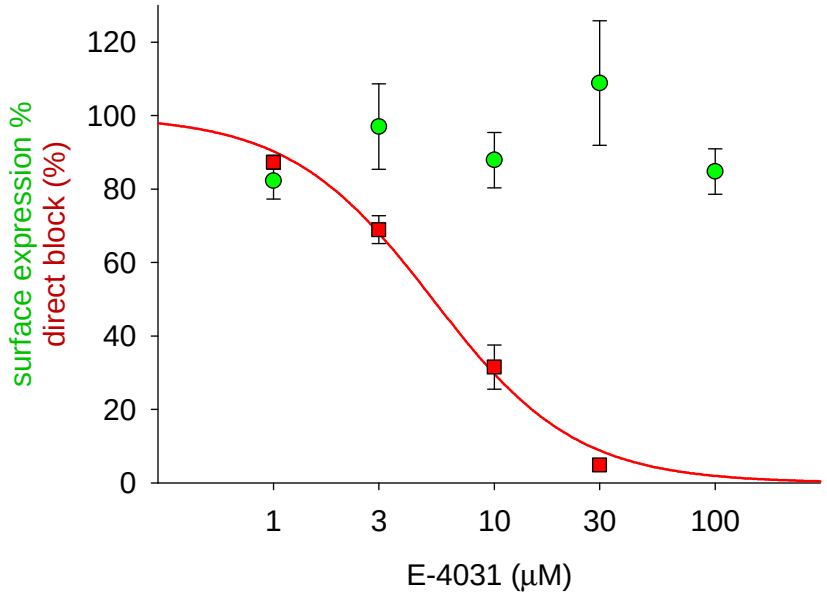
HEK293 hERG HA tag cells were continuously incubated at 37°C in a humidified atmosphere with 5% CO_2 (rel. humidity about 95%). Cells were plated on poly-L-lysine coated wells of a 96 well plate (white bottom / white walls). After at least 8 h the medium was removed and replaced with serum and antibioticum free medium containing the test items. Compounds were incubated over night (at least 16 h). All further steps were performed at RT. The wells were washed with PBS to remove compounds and fixed immediately with 4% Paraformaldehyde in PBS. Nonspecific antibody binding sites were blocked with 1% goat serum. To detect hERG proteins located in the plasma membrane a anti HA antibody, conjugated with a horseradish peroxidase was added together with SYBR green to stain the nucleus. After several wash steps the SYBR green fluorescence will be recorded to determine the amount of cells per well. PBS will be removed and replaced with SuperSignal®. luminescence will be recorded immediately.
For data analysis the luminescence signal will be normalized to the fluorescence signal to compensate potential cytotoxic effects of the test items. Wells with less than 25% cells compared to wells with vehicle treated cells were not used for analysis. After subtracting the cell number corrected vehicle luminescence measured in wells with non luminescent cells, luminescent values of compound treated cells were normalized to the luminescence of vehicle treated cells

3. VALIDATION

Eight compounds were tested to validate hERG trafficking effects. The tested compounds belonged to different effect classes: 1.) Direct hERG blockers that do not decrease (or even increase) the hERG surface expression: E-4031, Cisapride, Verapamil, 2.) Direct hERG blockers that decrease hERG surface expression: Bepridil, Fluoxetine, 3.) Compounds with no direct hERG block, but decrease of the hERG surface expression: Pentamidine, Ivermectine, Arsentrioxide.
Direct hERG block was tested in a standard patch-clamp experiment perfusing increasing concentrations of test item. Cells were not pre-incubated with test item.

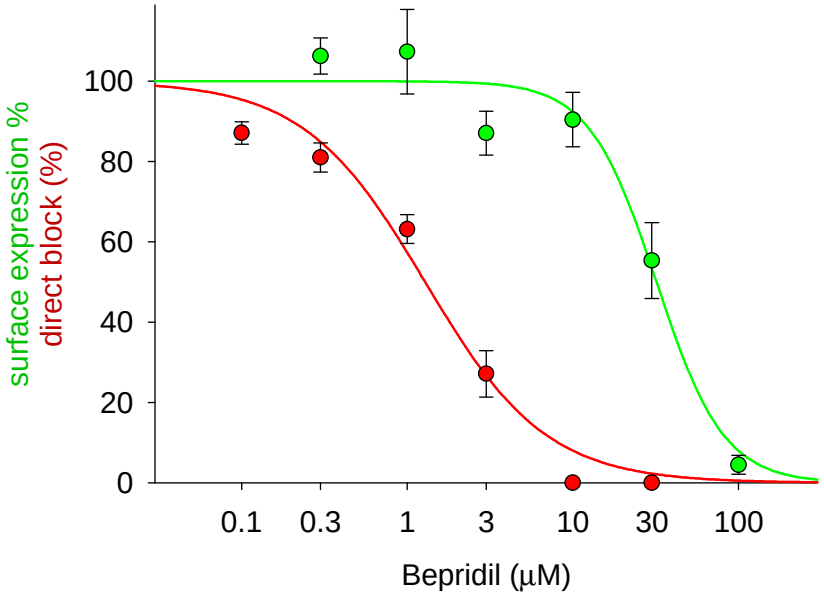
Group 1: Direct hERG blockers, no effect on hERG trafficking:

Compound	IC_{50} direct block	Maximal change of hERG surface expression	IC_{50} hERG surface expression
E-4031	5.26 nM	8.88% @ 30 nM	-
Cisapride	63 nM	-39.02% @ 30 µM	-
Verapamil	12.26 µM	121.71% @ 10 µM	-



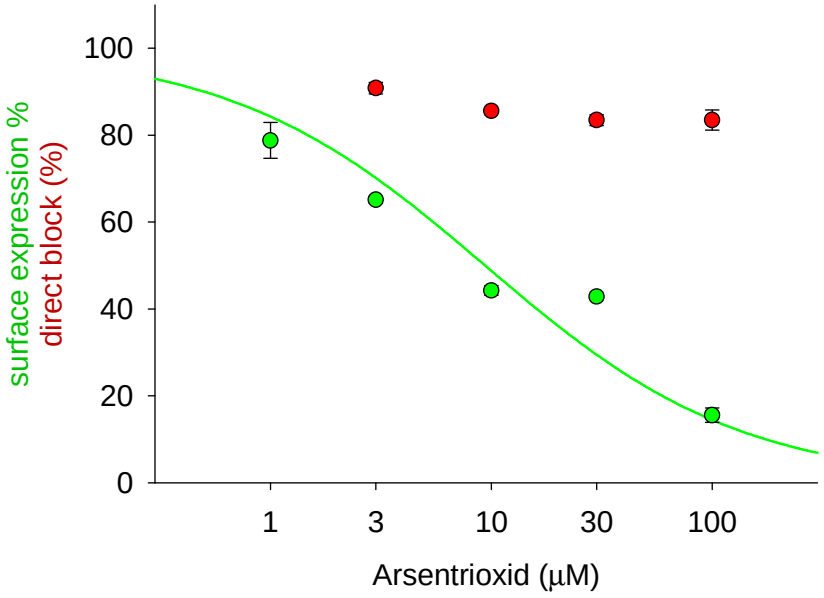
Group 2: Direct hERG blockers, with effects on hERG trafficking:

Compound	IC_{50} direct block	Maximal change of hERG surface expression	IC_{50} hERG surface expression
Bepridil	1.28 µM	-93.47% @ 100 µM	32.02 µM
Fluoxetine	1.23 µM	-28.52% @ 30 µM	90.03 µM



Group 3: No direct hERG blockers, with effects on hERG trafficking:

Compound	IC_{50} direct block	Maximal change of hERG surface expression	IC_{50} hERG surface expression
Pentamidine	Less than 30% inhibition	-54.78% @ 100 µM	80.07 µM
Arsentrioxide	Less than 30% inhibition	-84.42% @ 100 µM	9.39 µM
Ivermectine	Less than 30% inhibition	-95.45% @ 100 µM	5.29 µM



4. CONTACT INFORMATION

- B'SYS GmbH
  Technology Center Witterswil
  Benkenstrasse 254
  4108 Witterswil
  Switzerland

  Tel: +41 61 721 77 44
  Fax: +41 61 721 77 41
  Email: info@bsys.ch
  Web: www.bsys.ch