hERG safety pharmacology screening

Quality dose response data from hydrophobic substances

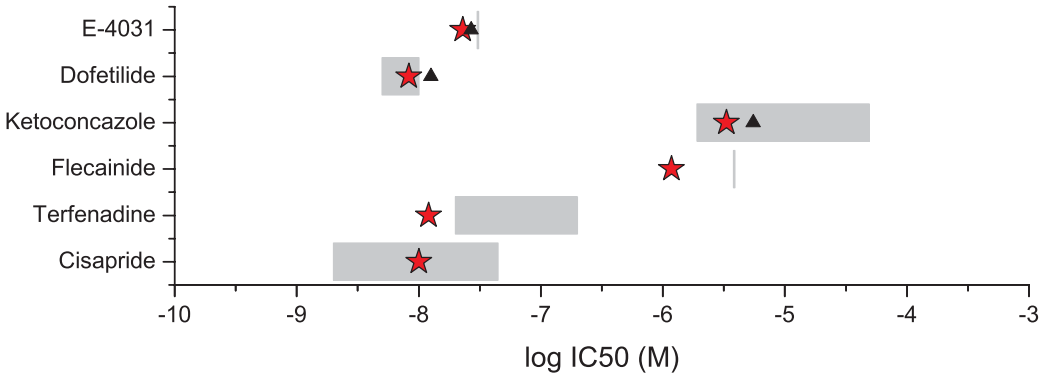
Ion channel: hERG

Cell type: CHO

Chip type: DF-8 Pro II

Data courtesy of bSys GmbH, Basel, Switzerland

Figure 1



Methods

Characterization of substance binding to the hERG channel is a critical step in cardiac safety pharmacology. The Dynaflow system and DF-8 Pro II chips were validated as a GLP hERG screening platform. The study covers a variety of inhibitory substances including hydrophobic compounds. Substance IC50 values obtained with the Dynaflow system show similar potency to those from conventional patch clamp and recording stability allowed for full dose response curves to be extracted from single cells. The performance of the Dynaflow system in this study demonstrates its suitability for conducting safety studies.

The results were obtained by eliciting hERG current responses with a voltage pulse protocol. CHO cells were stably transfected with cDNA encoding for the recombinant hERG potassium channel and kept under standard cell culture conditions. Cell suspension was achieved with standard procedure documented in the Cellectricon Cell Handling Document. All experiments were performed at room temperature.

Validation of IC50 values with Dynaflow

The IC50 values of substances tested with the Dynaflow system and DF-8 Pro II chips were in accordance with values obtained with bSys' conventional patch-clamp set up and values reported in the literature. **Figure 1** shows a bar diagram with a comparison of IC50 values. Stars represent the IC50 value recorded with the Dynaflow system. Triangles show the reference values from bSys' conventional patch clamp set up. Grey bars show the reference value range given in Redfern *et al.* 2003 [1]. For E-4031, the reference value is from Sorota *et al.* 2005 [2].

DF-8 Pro II used with hydrophobic substances

To validate the adsorption behavior of the DF-8 Pro II chips, Terfenadine, known as a highly hydrophobic substance, was used as a blocker of the hERG current. Cells were exposed to cumulative applications of 0.5, 1, 10, 30, 100 and 500 nM Terfenadine for 10 minutes at each concentration. Traces shown in **Figure 2** show the reproducibility of the data obtained with the Dynaflow system and DF-8 Pro II chips. Each plot is taken from a single cell, four cells in total were scanned on a single DF-8 Pro II chip.

Full dose-response curves from single cells

Current response of the hERG channel was elicited by the pulse protocol shown in **Figure 3B.** The voltage protocol was applied every 10 seconds. Typical current response of the hERG channel is shown in **Figure 3A**. The membrane and series resistance of all cells was controlled in a flow of extracellular buffer before starting the pulse protocol (Rm>750 MOhm, Rs 6-15 MOhm). Only peak amplitudes of tail currents of a minimum 200pA were taken into account for further experimental procedure. To generate a dose-response curve, antagonist was applied in 4-6 concentrations. Total recording time for each cell was at least 30 minutes and up to 60 minutes for slow acting substances. These recording times demonstrate that even with a slow acting ion channel like the hERG channel, a full dose response curve from a single cell could be extracted.

Once the pulse protocol was initiated, all cells were exposed to extracellular buffer for a minimum of 5 minutes, or until a stable base line current was reached. The peak amplitude of the control tail current was used to normalize the peak values of the current response at different antagonist concentrations. Measurement of the peak amplitude of the tail current was made in the range indicated by the two bars in **Figure 3A** and plotted over time as seen in the Dofetilide example in **Figure 3C**. In this graph, one can clearly see the time course of the block applying different concentrations of Dofetilide.

References

1. Redfern *et al* Cardiovasc Res. (2003) 58(1):32-45

2. Sorota *et al*. Assay and Drug Development Technologies (2005) 3(1):47-57.

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Figure 2

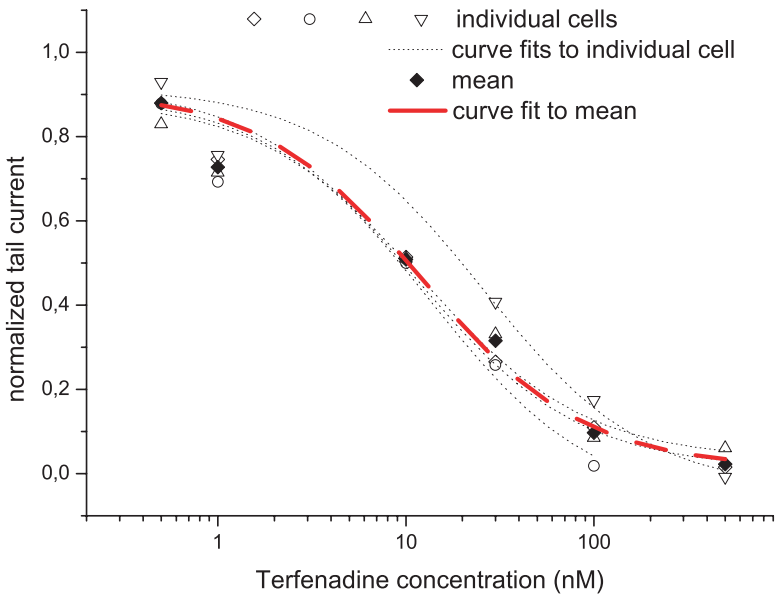
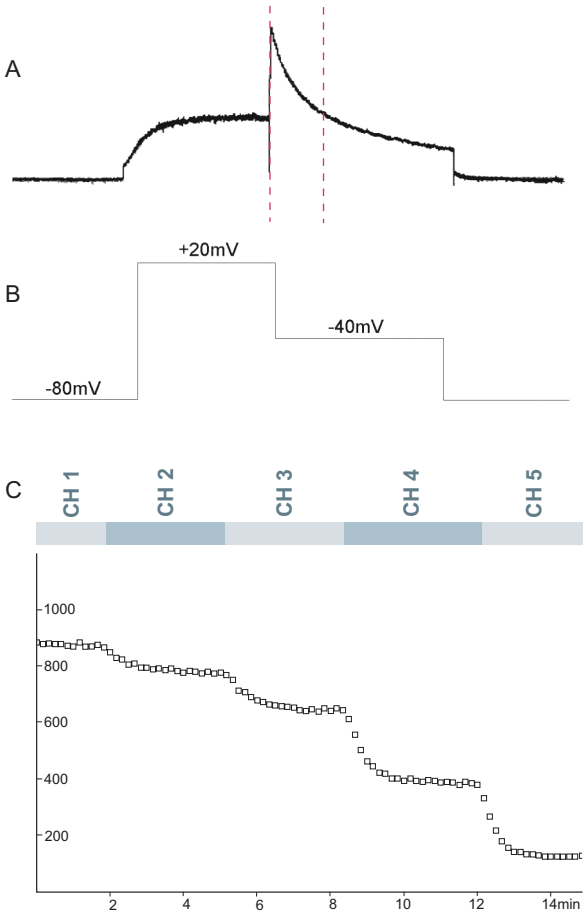


Figure 3



CH 1	buffer
CH 2	1 nM Dofetilide
CH 3	3 nM Dofetilide
CH 4	10 nM Dofetilide
CH 5	30 nM Dofetilide

CH = Dynaflow chip channel

Rev. 1. 11/2006