The inter-cell-line reproducibility of hERG assay using the whole-cell patch-clamping

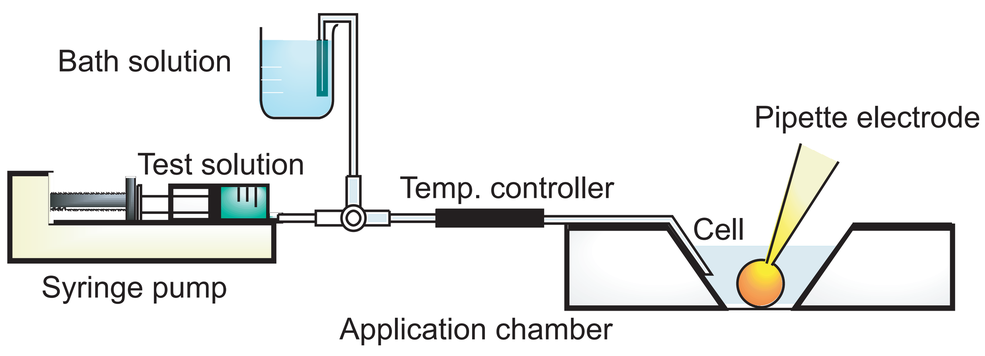
Yoshimi Katayama, Tomoko Tsuzaki, Kanae Abe, Satomi Tomizawa, Taku Izumi and Yuji Tsurubuchi

Drug Safety Testing Center Co. Ltd., Saitama, Japan

1. Introduction

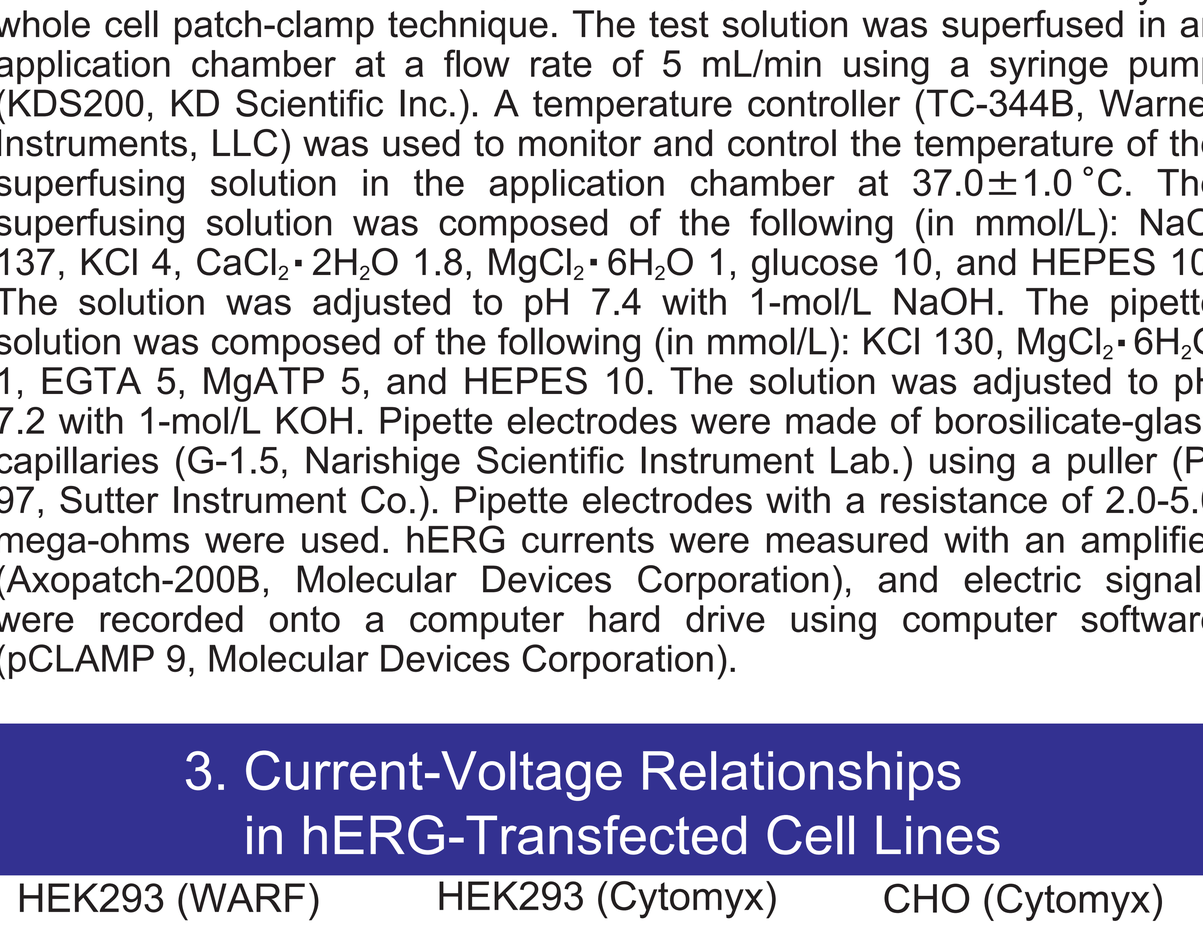
The whole-cell patch-clamp technique using cell lines transfected with human *ether-a-go-go*-related gene (hERG) has become more important recently for prediction of a potential risk of QT-interval prolongation that may lead to induction of life-threatening ventricular arrhythmia, *torsade de pointes*, and cardiac sudden death in clinical use of drugs. Numbers of recombinant cell lines have been developed recently for diverse purposes by various organizations; some of them are now commercially available for drug development including safety testing, such as the hERG assay. However, these cell lines had been established using various sorts of host cell lines as well as gene-transfection techniques. This study was conducted to investigate the inter-cell-line reproducibility in the hERG assay, which has not yet been well determined, using 3 cell lines that are commercially distributed by Wisconsin Alumni Research Foundation and Cytomyx Limited. Effects of 7 compounds that are known as hERG-channel blockers, including haloperidol, quinidine, astemizole, and E-4031, were evaluated using the whole-cell patch-clamp technique. IC_{50} values of the hERG-channel blockers were obtained and compared between cell lines in order to determine the reproducibility.

2. Materials and Methods



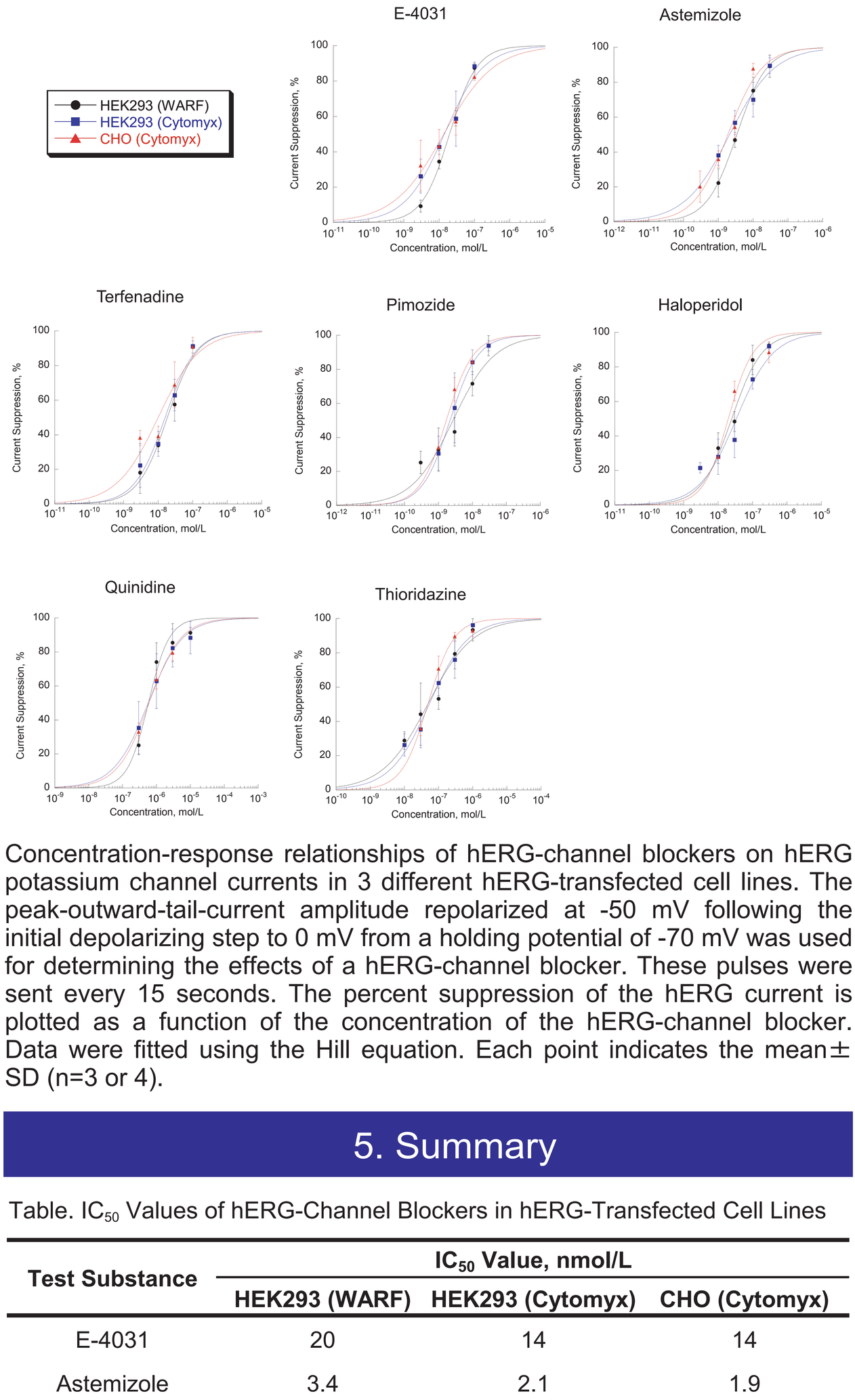
The schematic diagram of the test-substance application system. The inhibitory effects of hERG-channel blockers on the hERG potassium channel transfected in HEK293 cells or CHO cells were evaluated by the whole cell patch-clamp technique. The test solution was superfused in an application chamber at a flow rate of 5 mL/min using a syringe pump (KDS200, KD Scientific Inc.). A temperature controller (TC-344B, Warner Instruments, LLC) was used to monitor and control the temperature of the superfusing solution in the application chamber at 37.0±1.0 ˚C. The superfusing solution was composed of the following (in mmol/L): NaCl 137, KCl 4, $CaCl_2 \cdot 2H_2O$ 1.8, $MgCl_2 \cdot 6H_2O$ 1, glucose 10, and HEPES 10. The solution was adjusted to pH 7.4 with 1-mol/L NaOH. The pipette solution was composed of the following (in mmol/L): KCl 130, $MgCl_2 \cdot 6H_2O$ 1, EGTA 5, MgATP 5, and HEPES 10. The solution was adjusted to pH 7.2 with 1-mol/L KOH. Pipette electrodes were made of borosilicate-glass capillaries (G-1.5, Narishige Scientific Instrument Lab.) using a puller (P-97, Sutter Instrument Co.). Pipette electrodes with a resistance of 2.0-5.0 mega-ohms were used. hERG currents were measured with an amplifier (Axopatch-200B, Molecular Devices Corporation), and electric signals were recorded onto a computer hard drive using computer software (pCLAMP 9, Molecular Devices Corporation).

3. Current-Voltage Relationships in hERG-Transfected Cell Lines



Current-voltage Relationships obtained in 3 different hERG-transfected cell lines A: The current-voltage relationships for the steady-state currents. The outward currents at the end of the depolarizing step when the voltage protocol described in the inset was run were plotted as a function of the membrane potential of depolarizing pulses. The cell membrane of the hERG-transfected cell was held at -70 mV, depolarized to voltages from -50 to 40 mV for 0.75 to 3 seconds, and then repolarized to -50 mV for 0.75 to 3 seconds. B:. The peak tail currents during repolarizing steps when the same voltage protocol as described in A was run were plotted as a function of the membrane potential of depolarizing pulses. C: The peak tail currents during repolarizing steps when the voltage protocol described in the inset was run were plotted as a function of the membrane potential of repolarizing pulses. The cell membrane was depolarized to 0 mV for 0.75 second to activate and slightly inactivate the hERG potassium channels, and then clamped to various levels of potentials from -80 to 50 mV for 0.75 second.

4. Concentration-Response Relationships of hERG-Channel Blockers on the hERG Channel



Concentration-response relationships of hERG-channel blockers on hERG potassium channel currents in 3 different hERG-transfected cell lines. The peak-outward-tail-current amplitude repolarized at -50 mV following the initial depolarizing step to 0 mV from a holding potential of -70 mV was used for determining the effects of a hERG-channel blocker. These pulses were sent every 15 seconds. The percent suppression of the hERG current is plotted as a function of the concentration of the hERG-channel blocker. Data were fitted using the Hill equation. Each point indicates the mean± SD (n=3 or 4).

5. Summary

Table. IC_{50} Values of hERG-Channel Blockers in hERG-Transfected Cell Lines

Test Substance	IC_{50} Value, nmol/L		
	HEK293 (WARF)	HEK293 (Cytomyx)	CHO (Cytomyx)
E-4031	20	14	14
Astemizole	3.4	2.1	1.9
Terfenadine	18	16	10
Pimozide	2.7	2.2	1.7
Haloperidol	25	34	20
Quinidine	579	571	607
Thioridazine	48	53	49

Concentration-response data were fitted to the Hill equation, that is, % inhibition = 100 / {1 + (IC_{50} / [CB])^h}, where [CB], IC_{50}, and h represent concentration of a hERG-channel blocker, median inhibition concentration and the Hill coefficient, respectively (n=3 or 4). The IC_{50} value for the suppressive effect was calculated using a curve fitting program in KaleidaGraph 3.6 (Synergy Software).

6. Conclusion

The hERG potassium channel in 3 different hERG-transfected cell lines showed similar current-voltage relationships, therefore, it was decided to use the same voltage protocol for evaluating effects on known hERG-channel blockers. The deference of IC_{50} values for the respective hERG-channel blockers between the cell lines were little in this study. These results demonstrated the inter-cell-line reproducibility of the hERG assay is high and the assay would be reliable for prediction of drugs' potential risk of QT-interval prolongation.