# Patch-Clamp Studies in the Evaluation of Suppressive Effects of Drugs on the hERG Potassium Channel

Toshiyasu Honbo[1], Etsuko Kokubunji[1], Jiro Seki[1], Keiko Yonezawa[2], Mariko Amagai[2], Yoshimi Katayama[2], Kenji Tsuzaki[2] and Yuji Tsurubuchi[2]

[1] Fujisawa Pharmaceutical Co. Ltd., Osaka, Japan, [2] Drug Safety Testing Center Co. Ltd., Saitama, Japan

## 1. Introduction

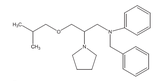
The Health and Environmental Sciences Institute, which is part of the International Life Sciences Institute (ILSI/HESI), has been building a database of the ion channel assay using a cultured cell line transfected with the human-ether-go-go-related gene (hERG) on standard drugs at two independent facilities. This work was conducted as a S7B activity of the International Conference for Harmonization (ICH) for establishing global guidelines for Safety Pharmacology Study. The data was reviewed at the ILSI Workshop in Washington, DC in June, 2003 and the ICH-Expert Working Group (EWG) Meeting of S7B and E14 in Brussels in July, 2003. Based on the results, it was revealed that there was an approximate 3-fold difference in $IC_{50}$ values of positive drugs between the two facilities. The inter-facility difference in sensitivity of the hERG assay has become a matter of concern.

This study was conducted by a third party to provide additional information and to encourage further discussions at ICH regarding the inter-facility differences in sensitivity of the hERG assay. The whole-cell patch-clamp technique using HEK293 cells transfected with hERG was used to evaluate the effects of Torsades de Pointes (TdP) positive and negative pharmaceuticals investigated by ILSI/HESI. Since the test procedure employed in this study is slightly different from those used in the evaluation by ILSI/HESI, a side-by-side comparison was conducted between these two procedures in order to determine if there is any influence on the results. Furthermore, other drugs such as E-4031, Sotalol, Quinidine, Lidocaine and Astemizole investigated by QT PRODACT in Japan were evaluated in order to provide reference data for discussion.

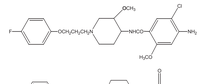
## 2. Test substances

A. Positive controls investigated by ILSI/HESI

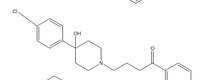
Bepridil



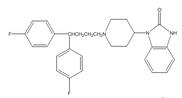
Cisapride



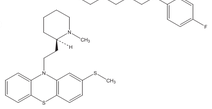
Haloperidol



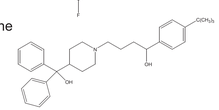
Pimozide



Thioridazine

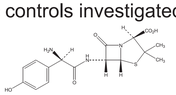


Terfenadine



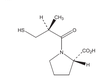
B. Negative controls investigated by ILSI/HESI

Amoxicillin

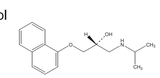


Aspirin

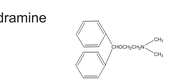
Captopril



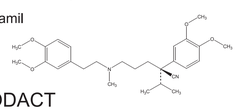
Propranolol



Diphenhydramine

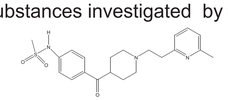


Verapamil

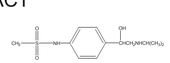


C. Test substances investigated by QT PRODACT

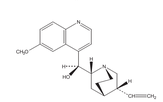
E-4031



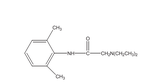
Sotalol



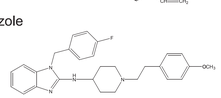
Quinidine



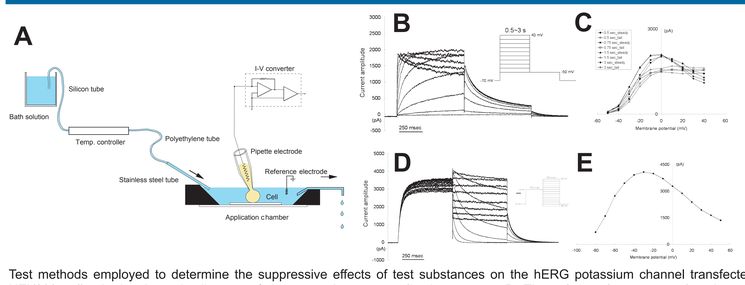
Lidocaine



Astemizole

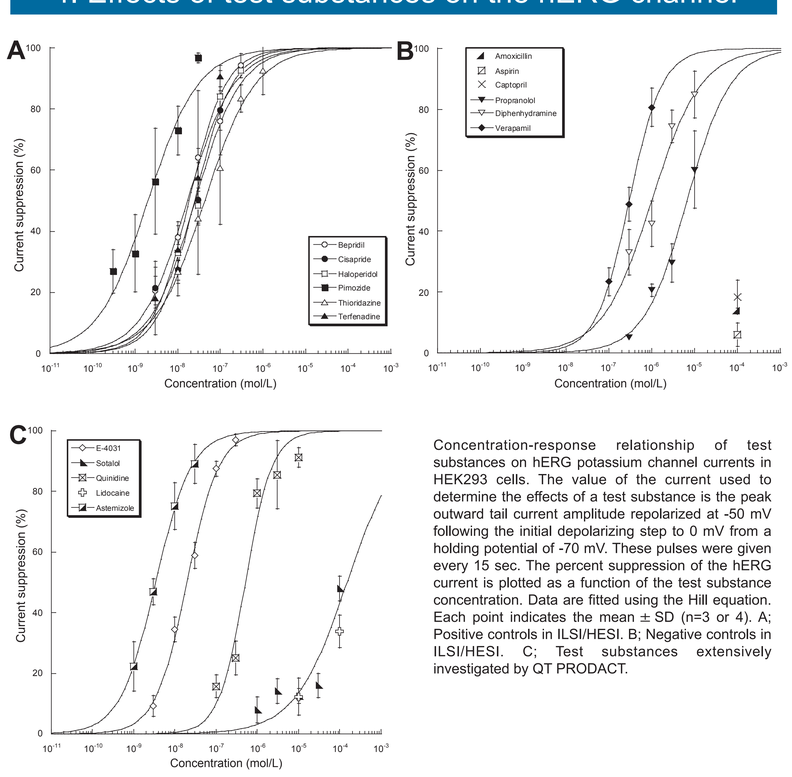


## 3. Methods



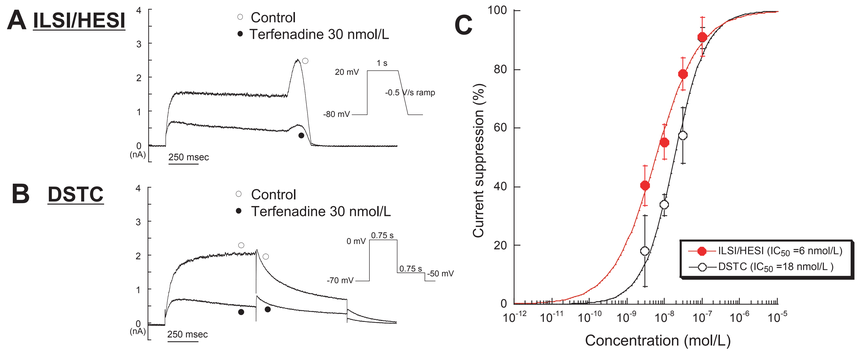

Test methods employed to determine the suppressive effects of test substances on the hERG potassium channel transfected in HEK293 cells. A; A schematic diagram of the test substance application system. B; The voltage clamp protocol and currents recorded from a hERG-transfected cell. The cell was held at -70 mV and depolarized to voltages between -50 to 40 mV for 0.5 to 3 sec, and then clamped to -50 mV for 0.75 sec. C; I-V plots of the hERG currents measured at the end of the depolarizing step and the peak tail currents when the voltage protocol described in C was run. D; The voltage clamp protocol and currents recorded from a hERG-transfected cell. The cell was depolarized to 0 mV for 0.75 sec to activate and slightly inactivate the hERG potassium channels, then repolarized to different voltages from -80 to 50 mV for 0.75 sec. E; I-V plots of the peak tail currents when the voltage protocol described in D was run. The temperature was maintained at 37±1˚C.

## 4. Effects of test substances on the hERG channel




Concentration-response relationship of test substances on hERG potassium channel currents in HEK293 cells. The value of the current used to determine the effects of a test substance is 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. These pulses were given every 15 sec. The percent suppression of the hERG current is plotted as a function of the test substance concentration. Data are fitted using the Hill equation. Each point indicates the mean ± SD (n=3 or 4). A; Positive controls in ILSI/HESI. B; Negative controls in ILSI/HESI. C; Test substances extensively investigated by QT PRODACT.

## 5. Comparison of test procedures between ILSI/HESI and DSTC




Effects of terfenadine on the hERG current under different test conditions. A; Superimposed current traces in an experiment following the test procedure used in the studies conducted by ILSI/HESI. The pulse command shown in the inset was given to the cell. Currents were elicited by a 1 sec depolarizing step to +20 mV from a holding potential of -80 mV, followed by a repolarizing ramp (-0.5 V/sec) to -80 mV. These pulses were given every 15 sec. Current records from a single HEK293 cell before (open circle) and 10 min after application of terfenadine at 30 nmol/L (closed circle) were superimposed. B; Superimposed current traces in an experiment using the standard test procedure employed at DSTC. The pulse command shown in the inset was given to the cell every 15 sec. Current records from a single HEK293 cell before (open circle) and 10 min after application of terfenadine at 30 nmol/L (closed circle) were superimposed. C; Effects of terfenadine on the concentration-response relationships for the hERG channel in HEK293 cells. The percent suppression of the hERG current under respective testing conditions is plotted as a function of the drug concentration. The $IC_{50}$ values of terfenadine under ILSI/HESI and DSTC procedures are estimated to be 6 and 18 nmol/L, respectively.

## 6. Summary

Table 1. Positive controls investigated by ILSI/HESI

| Test substance | $IC_{50}$ value (nmol/L) | | |
|---|---|---|---|
| | ChanTest * | Zenas * | DSTC |
| Bepridil | 24 | 84 | 17 |
| Cisapride | 21 | 31 | 26 |
| Haloperidol | 27 | 93 | 25 |
| Pimozide | 1 | 35 | 2 |
| Thioridazine | 74 | 256 | 43 |
| Terfenadine | 9 | 33 | 18 |

*: According to Data Summary Graphs of Cardiovascular Safety Subcommittee presented at ILSI Workshop in Washington, DC in June, 2003.

Table 2. Negative controls investigated by ILSI/HESI

| Test substance | $IC_{50}$ value (nmol/L) | | |
|---|---|---|---|
| | ChanTest * | Zenas * | DSTC |
| Amoxicillin | N.A. | N.A. | >100000 |
| Aspirin | N.A. | N.A. | >100000 |
| Captopril | N.A. | N.A. | >100000 |
| Propranolol | 8200 | 2800 | 6658 |
| Diphenhydramine | 1900 | 5700 | 997 |
| Verapamil | 180 | 252 | 299 |

N.A. : Not Applicable, $IC_{50}$ value could not be identified
*: According to Data Summary Graphs of Cardiovascular Safety Subcommittee presented at ILSI Workshop in Washington, DC in June, 2003

Table 3. Test substances investigated by QT PRODACT

| Test substance | $IC_{50}$ value (nmol/L) |
|---|---|
| | DSTC |
| E-4031 | 20 |
| Sotalol | 149860 |
| Quinidine | 514 |
| Lidocaine | >100000 |
| Astemizole | 3 |

## 7. Conclusion

The $IC_{50}$ values for suppressive effects of test substances on the hERG channel obtained in this study were all similar to either or both of the two data previously presented by ILSI/HESI. The difference of the test procedures between ILSI/HESI and DSTC for the evaluation resulted in little variance of $IC_{50}$ values of terfenadine. These results suggest that the inter-facility difference in sensitivity of the hERG assay is limited and the assay would be reliable as a preclinical global standard to determine the QT prolongation potency of drugs.

## Acknowledgement

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