Correlation of hERG Screening Data Between Conventional and Automated Patch-Clamp Systems

Tomoko TSUZAKI, Kenji TSUZAKI, Yoshimi KATAYAMA and Yuji TSURUBUCHI

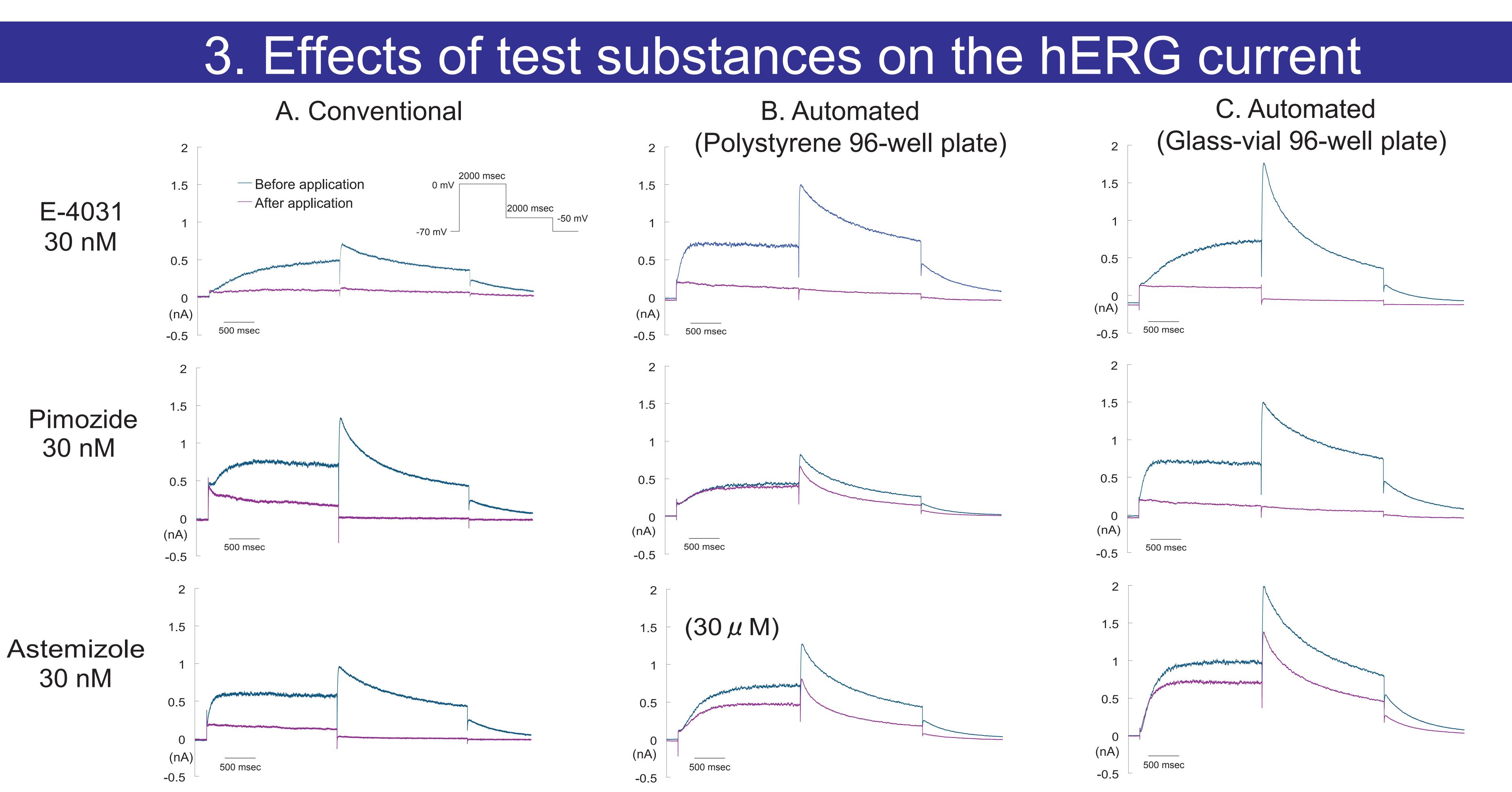Drug Safety Testing Center Co., Ltd., Saitama, Japan

1. Introduction

The whole-cell patch-clamp technique using human ether-a-go-go-related gene (hERG) transfected cell lines is one of the most important assays in safety pharmacology studies to predict a drug's potential risk of *torsade de pointes*. However, the patch-clamp experiment is very time consuming, although it is more reliable than some other assays. The automated patch-clamp system is considered to be convenient and reliable to screen a number of drugs in an early stage of development with short turn-around time. In this research, correlation of data between conventional patch-clamping and automated patch-clamp system, PatchXpress 7000A, in terms of the hERG-current-suppression effects of positive-control substances was determined using hERG-transfected HEK293 cells.
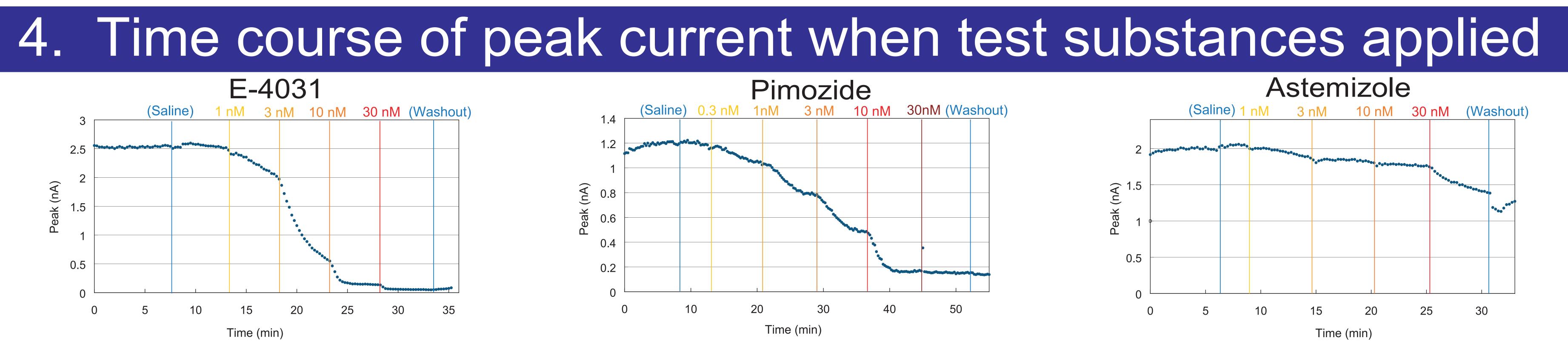
2. Methods

A. Conventional Patch-Clampling   B. Automated Patch-Clamping

Test methods for determination of the suppressive effect of test substances on hERG potassium channels transfected in HEK293 cells. A: A schematic diagram of test substance application in the conventional patch-clamp system. B: A schematic diagram of test substance application in the automated patch-clamp system. All experiments were performed at room temperature.

3. Effects of test substances on the hERG current

Effects of test substances on the hERG current in the conventional and automated patch-clamp systems. A: The hERG current recorded before and after application of positive-control substances in the conventional patch-clamping. The pulse command shown in the inset was injected to the cell every 15 seconds. Current records from a single HEK293 cell before and 10 minutes after application of the respective positive-control substance are superimposed. B: The hERG current recorded before and after application of positive-control substances in the automated patch-clamp system. Polystyrene plates were used to store test-substance solutions. The concentration of astemizole (30 μM) was higher than that in B and C. C: The hERG current recorded before and after application of positive-control substances in the automated patch-clamp system. Glass-vial plates were used to store test-substance solutions.

4. Time course of peak current when test substances applied

Time course of peak-tail-current suppression in the absence and presence of test substance. Test substances were cumulatively applied from lower to higher concentrations in the automated patch-clamp system. Glass-vial plates were used to store test-substance solutions prior to application. E-4031 and pimozide showed time- and concentration-dependent hERG-current suppression. In contrast, the time- and concentration-dependency of suppression induced by astemizole observed in the automated patch-clamp system was not clear and the suppressive effect was much less than that in the conventional patch-clamping.

5. Summary

Test Substance	Conventional	IC$_{50}$ Value (nM) Automated (Polystyrene plate)	Automated (Glass vial)
Haloperidol	23	110	45
Pimozide	1.3	120	3.4
E-4031	40	27	56
Quinidine	750	1500	-
Bepridil	28	530	120
Terfenadine	16	220	85
Diphenhydramine	5000	4400	-
Verapamil	300	830	320
Thioridazine	92	1000	340
Sotalol	450000	700000	220000
Astemizole	1.4	54000	56

6. Conclusion

On most of the test substances examined in this research, weak correlation was observed between the conventional and automated patch-clamp systems, when polystyrene plates were used. In contrast, the equivalent results were obtained on most of the substances between these systems, when glass-vial plates were used to store test-substance solution in the automated patch clamp system. This fact is considered to result from adsorption into plates to store test-substance solutions. However, the suppression effect was inconsistent between the automated patch-clamp system and conventional patch-clamping in some positive-control substances even when, test-substance solutions were stored in glass-vial plates in the automated patch-clamp system. Among those test substances, astemizole showed the most distinct difference.

To prevent differences in evaluation between the conventional and automated patch-clamp systemsas much as possible, it is recommended to select relevant compound plate to store test-substance solutions. For further studies on the automated patch-clamp systems, it would be expected to determine the voltage-clamp protocols used for the evaluation and vehicle to prepare the test-substance solution.