Determination of IC_{50} values for hERG blockers using an automated patch-clamp screening system

Kenji Tsuzaki[1], Niels J Willumsen[2,3], Morten R Sunesen[3] and Yuji Tsurubuchi[1]

[1] Drug Safety Testing Center Co. Ltd., Saitama, Japan,

[2] August Krogh Institute, University of Copenhagen, Copenhagen , Denmark,

[3] Sophion Bioscience A/S, Ballerup, Denmark

1. Introduction

Screening new drug candidates for unforeseen and potentially fatal side-effects on cardiac ion channels has increased the need for precise and efficient electrophysiological testing with higher throughput than is possible with conventional patch-clamp. We report the determination of IC_{50} values for inhibition of the human ether-a-go-go-related gene (hERG) potassium channel from two studies using an automated 16-channel patch-clamp system, QPatch 16: (A) a test of 8 known reference compounds conducted at Sophion Bioscience to compare the results to the reference data established at Drug Safety Testing Center (DSTC) by the conventiona pacth-clamping, and (B) a study of 32 unknown compounds tested by Sophion Bioscience in a blind test to compare the results to the conventional patch-clamping data provided by the supplier of the compounds. The QPatch 16 has the capacity to provide 250-1200 whole-cell experiments ('data points') per working day, i.e. 8 hours. In both studies we analyzed the correlation between IC50 data obtained with the QPatch-16 and data obtained with conventional patch-clamp.

2. Materials and Methods

CHO cells expressing hERG channels were employed. The extracellular Na+ Ringer solutions consisted of (in mM): : 145 NaCl, 4 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES (pH 7.4), and 10 glucose. The intracellular K^+ Ringer solutions consisted of (in mM): 120 KCl, 5.4 KCl_2, 1.8 $MgCl_2$, 10 KOH/EGTA, Na_2-ATP, and 10 HEPES (pH 7.2). Reference compounds were acquired from commercial resources. Unknown test compounds for the blind study were kindly provided by Aventis (Bridgewater, NJ, USA). rBeKm-1 was from Alomone Labs, Israel. In manual patch-clamp experiments the cells were kept at a membrane potential (Vm) of -80 mV. Every 15 seconds Vm was depolarized to +20 mV for 2000 ms, and subsequently Vm was partly repolarized to -50 mV (2000 ms) at which potential the maximal tail currents were measured.In the QPatch experiments Vm was clamped to -60 mV, depolarized to +20 mV for 2000 ms, and subsequently repolarized to -60 mV at which potential the maximal tail currents were measured.

In study A, 4-5 concentrations were used (N=4-5 measurements per concentration) for automated as well as manual determinations. In study B, 5 compound concentrations were used (N=2-5 per measurements per concentration). In the retest (see below) 4 compound concentrations (N=4-5, 240 data points) were used. Figure 1 shows an original hERG current trace (top), effect of increasing blocker concentrations over time (middle), and the complete concentration-response graph (bottom) as viewed in the QPatch analysis software.

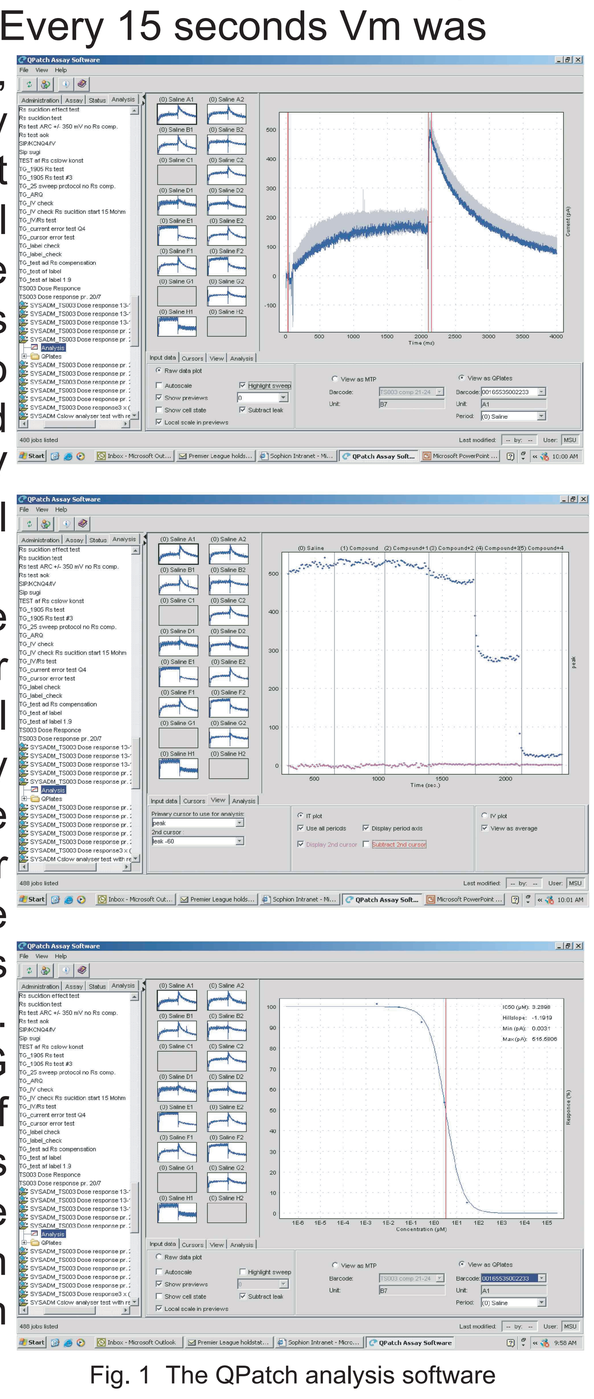

Fig. 1 The QPatch analysis software

3. Study A: Correlation of IC_{50} Values of Known Reference Compounds

This study included 8 compounds with known hERG blocking ability: pimozide, astemizole, terfenadine, haloperidol, bepridel, E-4031, verapamil, and quinidine. Figure 2 shows the QPatch IC_{50} values compared to the IC_{50} values obtained with conventional patch-clamp by DSTC. This study was done in approximately 8 hours (one working day). Figure 2 also includes IC_{50} data potent hERG blocking scorpion venom rBeKm-1. A strong correlation exists between the two sets of data. Figure 3 demonstrates that the rank order of potency determined with either the conventional or the automated experiments is practically identical.

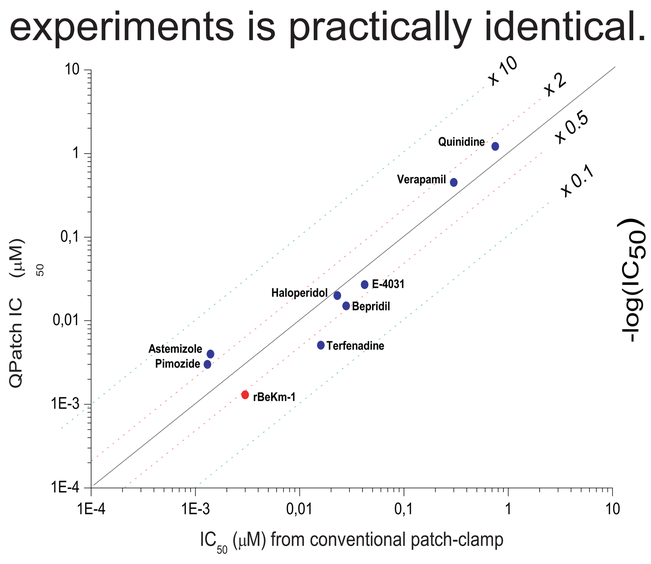

Fig. 2 Correlation of IC_{50} values of known reference compounds between conventional patch-clamping and QPatch system

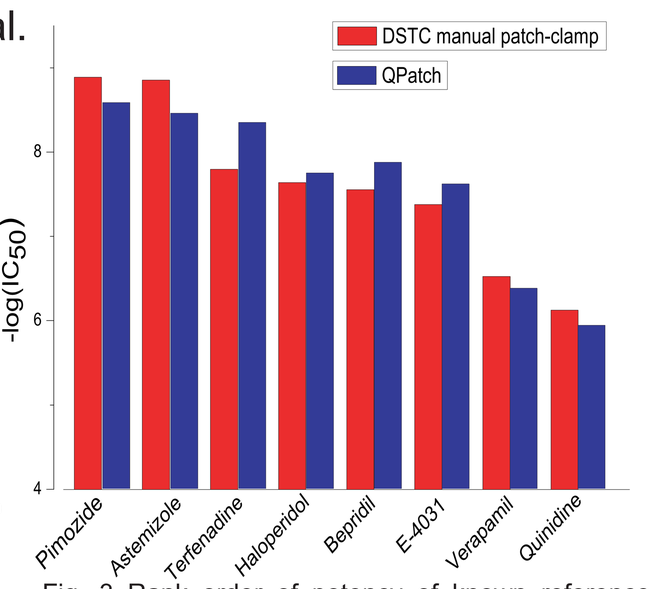

Fig. 3 Rank order of potency of known reference compounds determined with either the conventional or the automated experiments

4. Study B: Correlation of IC_{50} Values of Unknown Compounds in Blind Test

Thirty-two unknown compounds were received, dissolved in stock solutions in plastic microtitre plates, and applied to the cells on the QPatch 16. In Figure 4 the resulting IC_{50} values are compared with IC_{50} values obtained with manual path-clamp. It is seen that a population (blue oval) of QPatch IC_{50} data points appear to be systematically increased relative to results from manual patch-clamp. The average deviation factor was 2.2.

It was rationalized that a likely cause of the discrepancy between the two data sets could be non-specific binding of 'sticky' compounds to the plastic surfaces in the microtitre plates (in the QPatch 16 the compounds are minimally exposed to plastic surfaces). To test this hypothesis, 11 of the compounds with QPatch IC_{50} values deviating most significantly from the manually determined values were retested in a study in which they had not been in contact with any plastic surfaces (i.e., glass-coated microtitre plates were used to hold compounds). This caused all but one IC_{50} value to be reduced.

The combined results of the original test and the retest are shown in Figure 6. Figure 7 depicts the rank order of potency for the compounds with manually determined IC_{50} values < 1 μM (N=13). Black data points represent the original test; blue data points represent the retest. Also rBeKm-1 is included (red data point). The average deviation was a factor of 1.15 upon the retest.

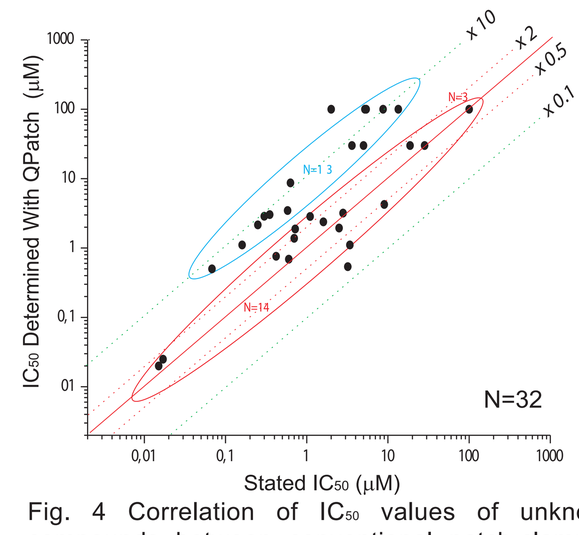

Fig. 4 Correlation of IC_{50} values of unknown compounds between conventional patch-clamping and QPatch system using

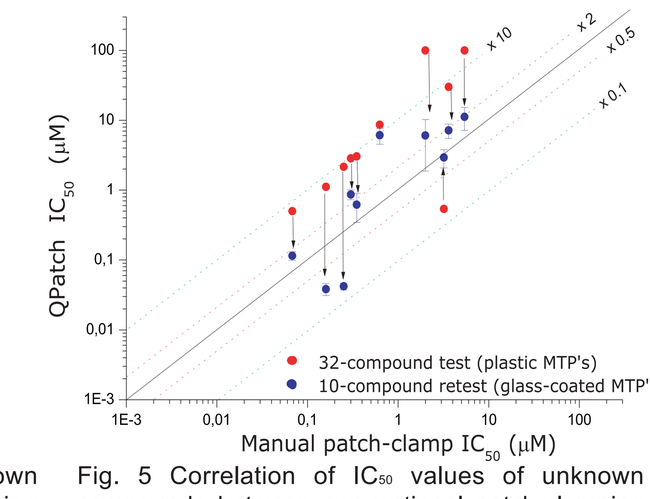

Fig. 5 Correlation of IC_{50} values of unknown compounds between conventional patch-clamping and QPatch system using glass-coated microtitre plates

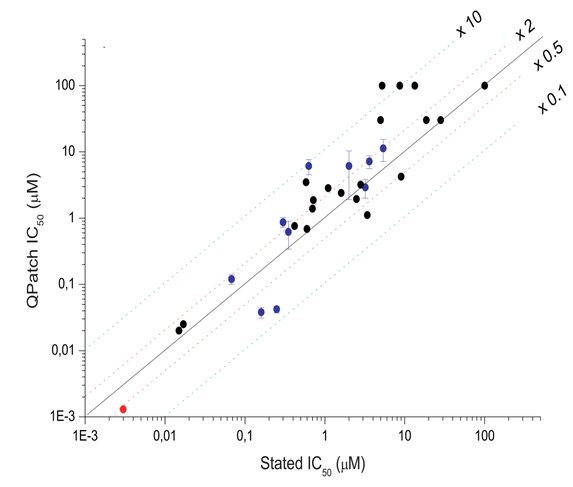

Fig. 6 Combined results of the original test (Fig. 4) and the retest (Fig. 5)

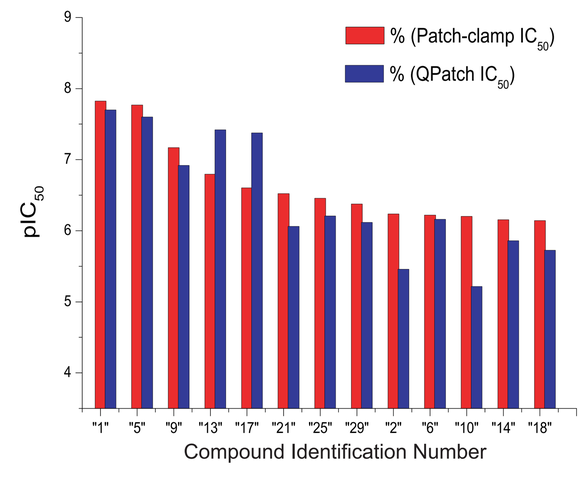

Fig. 7 Rank order of potency of unknown compounds determined with either the conventional or the automated experiments

5. Conclusion

The correlation between data obtained with automated patch-clamp (QPatch 16) and manual patch-clamp was high. Thus, 59% of the IC50 values in study B deviated less than two-fold, and 97% deviated less than ten-fold. We conclude that the electrophysiological data obtained with the QPatch automated patch-clamp system are precise. The problem with overestimation of IC_{50} values due to 'sticky compounds' was reduced because the compounds in the QPatch system are minimally exposed to plastic surfaces. Finally, the QPatch provides a substantial increase in throughput as compared to conventional manual patch-clamp.

The complete QPatch™ screening station