Optimization of hERG screening using IonFlux HT automated system

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The human Ether-à-go-go related gene (hERG) represents the molecular correlate of the IKr current, one of the several potassium currents involved in cardiac action potential repolarization. It is widely accepted that for most compounds, the primary mechanism for QT interval prolongation is direct inhibition of the IKr current. Therefore, compound testing for hERG inhibition is critical in the early safety evaluation process. To access directly to effects of compounds on channel function, only **electrophysiological technique** is required. The most classical technique is the conventional patch-clamp but the output is limited. Planar automated path-clamp systems have been developed to increase this output including IonWorks, Qpatch and Patchxpress. Because the introduction of planar system was drastically increased the cost of hERG test, the development of the new technology drives a tradeoff between data quality and cost of production. Here we describe the ability of **IonFlux HT® automated patch-clamp system** (Fluxion Biosciences) to study hERG current inhibition versus an other automated patch-clamp system (patchXpress) and conventional patch-clamp.

Cell culture

Recombinant Chinese Hamster Ovary (CHO) cell line expressing the human ERG potassium channel (Cytomyx CYL3038) was cultured in glutamax DMEM/F12 medium (Gibco 11320) containing 10 % fetal bovine serum and 1% G418 (Gibco 10131) in a humidified, 5% CO2 atmosphere. Cells were either grown at 37°c then transferred to a 30°C incubator at least 24 hrs before the experiments. For experiments, cells reaching 70-80% confluence were released from flasks using Accumax (Sigma ; 1:4) and after washing and gentle trituration, a suspension of 2 – 5 million cells per mL was introduced in extracellular solution for IonFlux HT or patchXpress experiments, or the cells were sown at 5000 cells by 35 mm dish for conventional patch-clamp.

Solutions and compounds

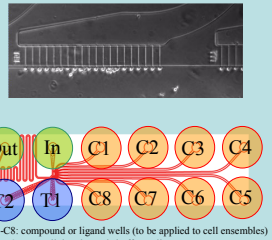
The extracellular solution contained NaCl, 138 mM; KCl, 4 mM; CaCl2, 1.8 mM; MgCl2, 1 mM; glucose, 5.6 mM, HEPES, 10 mM. The pH was adjusted to 7.3 with NaOH and the osmolarity was adjusted to 285 mOsm. The internal solution contained KCl, 60 mM; KF, 70 mM; NaCl 15 mM; HEPES, 5 mM; EGTA(K) 5 mM. The pH was adjusted to 7.25 with KOH and the osmolarity was adjusted to 290 mOsm.
HERG blocker compounds were purchased from Sigma. SAR compounds are homestead of sanofi-aventis. All compounds were first dissolved in 10 or 30 mM DMSO then diluted in doses series in DMSO before final dilution in external solution. The final DMSO concentration was 0.1 %.

Experiment protocols

IonFlux HT experiments. Each well of the IonFlux HT well plate consumable was loaded with 90 µL of internal solution, compound, or cell suspension. IonFlux HT plates look and handle exactly like standard 384 well plates, but the plate bottom is replaced by a microfluidic network that connects to the wells in a repeating pattern across the plate. After loading the plate, all flow control steps are controlled by the instrument, including cell trapping, seal formation, whole cell break-in, compound application, and washing. The IonFlux HT system includes 64 fully-featured amplifiers. All 64 recording channels are simultaneously applied with the specified voltage command waveforms. The system utilizes 20-cell ensembles for each amplifier channel to improve data consistency and success rates. For recording hERG currents, cell ensembles are voltage clamped at a holding potential of -80 mV, and seal resistances are constantly monitored by a small step to -100 mV. HERG channels were activated at +50 mV (800 ms), and the outward tail current at -50 mV is measured by subtracting a baseline reading at -50 mV before activation from the peak outward tail current at -50 mV after activation. A step to -120 mV (800 ms) after the -50 mV repolarization step is also included for recovery of hERG channels from inactivation. The voltage protocol is applied every 6 s. Leak current is compensated online using two pairs of small pulses (-80 mV to -100 mV 50 ms/50 ms). Ionic currents are sampled at 5 kHz.

PachXpress experiments. Cells distributed in 16 wells containing sealchip. Like for IonFlux HT, the PatchXpress controls every stage except that the cells and compounds automatically added to the wells by a pipette. The hERG current was activated in response to voltage steps (2 s) from a holding potential of - 80 mV to a test potential of + 20 mV followed by a repolarization to - 100 mV. Voltage steps are applied at 20 s intervals. No leak current compensation is applied. HERG current is measured as the tail deactivating current at - 100 mV.

Conventional patch-clamp experiments. Glass micropipette electrodes with tip resistances of 3-5 Mohms are connected to a voltage clamp amplifier. No leak subtraction is applied. Current signals are filtered (1 kHz) then acquired using an analogue-to-digital converter connected to a PC and analysed using Pclamp software. Same electrophysiolocal protocol as Patchxpress is led. Compounds are continuously superfused on the recorded cells and experiments are made at 35°C.



C1-C8: compound or ligand wells (to be applied to cell ensembles)
T1, T2: Intracellular electrode buffer wells
IN: Cell suspension inlet well
OUT: Waste well
P1 to P32: Pattern number, 1-32

IonFlux HT system

The IonFlux HT system utilizes a "plate reader" format to simplify workflow and increase throughput and uses 64 multiplexed amplifiers.
IonFlux plates include a microfluidic network for cell introduction, trapping, seal formation, and compound application. The IonFlux HT reader contains an interface that connects to the plate, providing pneumatic flow control and electrodes for continuous recording.
The IonFlux plate records from a 20-cell ensemble in parallel, providing the best combination of consistency, success rate, and recording fidelity. Active fluidic control is utilized to introduce cells, move them to the trapping area, and position the cells for optimal trapping.

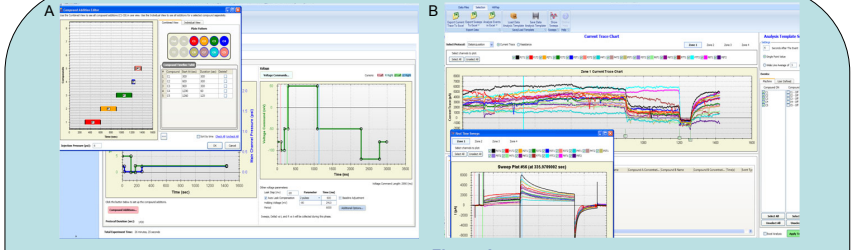
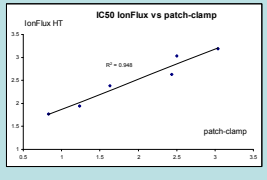


Figure A shows the drug application protocol and the waveform of the voltage protocol.
Figure B shows that the IonFluxApp software allows to superimpose online the time course amplitude of all or a part of current or of resistance by zone. The bottom part of the graph illustrates a typical screenshot of hERG current recorded during runtime with DMSO control.
Figure C shows five superimposed traces recorded in the same drug condition. This protocol includes 5 min successive perfusion of DMSO control, 1 µM compound, 10 µM compound, then positive control and washout, reached a mean success rate of about 70% *vs* 50% with patchXpress. A statistical analysis with 3 plates tested on the same day and with the same compound showed slight variability between plates and a standard deviation of about 10%.

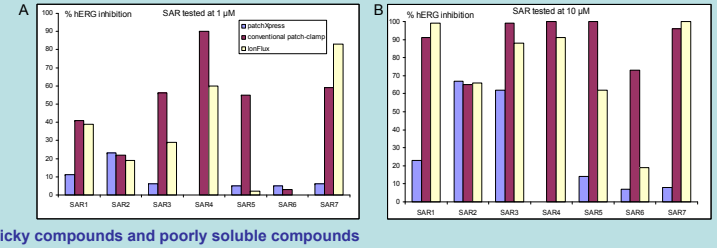
A

Compound	IonFluxHT IC50 (nM)	Manual Patch-Clamp IC50 (nM)	PatchXpress IC50 (nM)
Risperidone	424	274	553
Quinidine	1530	1100	2560
Astemizole	58	7	28
Cisapride	243	43	69
Dofetilide	87	17	188
Verapamil	1090	319	473

B



Calculated IC50 values of known hERG blockers were determined with IonFlux HT (n = 9 to 14). IC_{50} values on conventional patch-clamp and patchXpress are indicated for comparison in the table A. The logarithm of IC_{50} on IonFlux HT and conventional patch-clamp are plotted in Figure B. The IC_{50} values with the different systems used showed a clear correlation and similar range of values.



Sticky compounds and poorly soluble compounds

During compounds screening on hERG channel, we have identified a small part of compounds which had lower inhibition activity on patchXpress than on conventional patch-clamp. These compounds were sticky compounds (SAR1 to SAR4) or poorly soluble compounds (SAR5 to SAR7). These compounds were tested on IonFlux HT and the hERG percents of inhibition at 1 µM (A) and 10 µM (B) were compared to conventional patch-clamp and patchXpress. These graphs showed that at 1 and 10 µM sticky compounds acted similarly on IonFlux HT and conventional patch-clamp highlighting the well-suited drug application process of IonFlux HT for potential sticky compounds. For poorly soluble compounds, effects on hERG current with IonFlux HT were better highlighted with conventional patch-clamp. Since it is possible to test compounds at 37°C with IonFlux HT, it would be interesting to increase temperature to promote compound solubility and efficiency effects as for conventional patch-clamp experiments.

The evaluation of the IonFlux HT showed that this new microfluidic system allows a good success rate (about 70%) and reproducibility of results with a much higher through-put than conventional patch-clamp and patchXpress. The IonFlux HT allows performing accurate cardiovascular safety screening with a reduced cost.

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