

Identification of cardiac liability in drug discovery using the Port-a-Patch

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Summary

In 2013 the Cardiac Safety Research Consortium (CSRC), the Health and Environmental Sciences Institute (HESI), and the US Food and Drug Administration (FDA) proposed a paradigm to improve assessment of the proarrhythmic risk of therapeutic compounds. This paradigm, the Comprehensive In-vitro Proarrhythmia Assay (CiPA), was introduced to provide a more complete assessment of proarrhythmic risk by evaluating and implementing currently available high throughput methods. An important part of this is the electrophysiological evaluation of hERG, and also other cardiac channels including $\text{Na}_v1.5$ and $\text{Ca}_v1.2$. The Q&A draft from August 2020¹ describes how nonclinical assays such as patch clamp can be used as a part of an integrated risk assessment prior to first-in-human studies, and in later stages of clinical development.

Following up on hERG and $\text{Na}_v1.5$ best practices and calibration standards which have been published recently²⁻⁴ on automated patch clamp devices, we show here cardiac ion channel recordings from human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) or overexpressing cell lines generated with the world's smallest patch clamp setups: Port-a-Patch and Port-a-Patch mini. Recordings at RT or physiological temperature of hERG recorded from HEK cells, and peak or late I_{Na} current recorded from iPSC-CMs or CHO cells are shown. I_{Na} -Late was activated by ATX-II and blocked by ranolazine, I_{Na} -Peak was blocked by tetracaine in a concentration-dependent manner, and hERG was blocked by increasing concentrations of dofetilide.

Results

One goal of the CiPA initiative is to determine the requirements needed to deliver robust, reliable and reproducible ion channel data in a screening environment using high and medium throughput automated patch clamp (APC) systems like the Syncro-Patch 384 or the Patchliner, low throughput systems like the Port-a-Patch family or manual patch clamp. Figure 1 shows the different aspects of the CiPA initiative for a multi-faceted approach to safety liability testing of compounds.

Comprehensive *in Vitro* Proarrhythmia Assay: Four Components

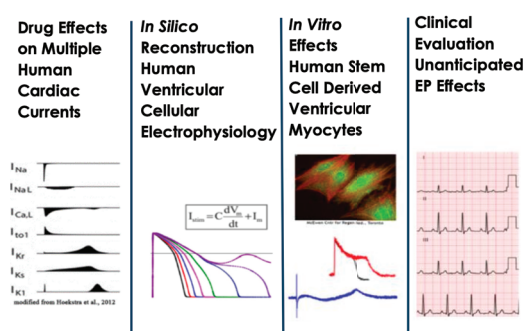


Figure 1: The different aspects of the CiPA initiative for a more comprehensive assessment of cardiac safety testing of compounds.

Application Note

Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) are becoming of increasing interest in cardiac safety testing due to their relative abundance, their human origin and their recapitulation of native human cardiomyocyte behavior. The late current ($I_{Na-Late}$), recorded during the ramp phase of the voltage protocol (Figure 2A), was enhanced by ATX-II and blocked by ranolazine (Figure 2B, C).

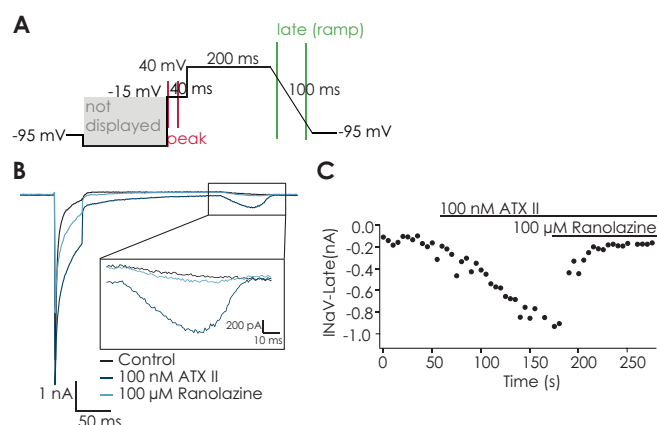


Figure 2: $I_{Na-Late}$ recorded from hiPSC-CMs. **A** Voltage protocol used for $Na_v1.5$ peak and late recordings. **B** $I_{Na-Late}$ and Late currents were recorded using the protocol shown in A. $I_{Na-Late}$ is shown enlarged in the inset. $I_{Na-Late}$ was enhanced by ATX-II and blocked by ranolazine. **C** Timecourse of the experiment.

Peak $Na_v1.5$ current ($I_{Na-Peak}$) was recorded from CHO cells expressing $Na_v1.5$ on the Port-a-Patch mini. First currents were recorded in response to increasing voltage steps from -60 to +60 mV from a holding potential of -100 mV. A current-voltage plot was constructed and the data fit with a Boltzmann equation revealing a V_{half} of activation of -32 mV (Figure 3A). Using a single voltage step protocol to -15 mV repeated every 10 s, $Na_v1.5$ -mediated currents were blocked by increasing concentrations of tetracaine (Figure 3B, C). The concentration response curve for an average of 6 cells was constructed (Figure 3D) and an IC_{50} of $25.5 \pm 5.7 \mu M$ was calculated. This is in good agreement with the range found in the literature^{5,6}, although block of Na_v channels by local anaesthetics is strongly state-

dependent, binding with stronger affinity to the open and inactivated states compared with the resting state⁶. This makes direct comparison with literature values complex.

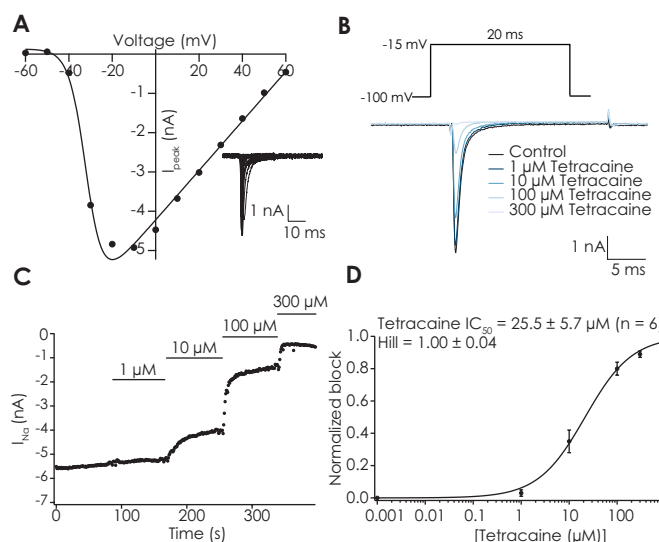


Figure 3: $I_{Na-Peak}$ recorded from CHO cells. **A** Current-voltage plot of an example CHO cell expressing $Na_v1.5$. Responses to increasing voltage steps are shown. **B** Block of $Na_v1.5$ by increasing concentration of tetracaine. The top panel shows the single step voltage plot and the bottom panel the traces from an example cell. **C** Corresponding timecourse of the experiment. **D** Concentration response curve for tetracaine for an average of 6 cells recorded on the Port-a-Patch mini.

Validation studies confirm that testing the effect of compounds on an increased number of human cardiac ion channel currents including I_{Na} ($Na_v1.5$ peak and late current) as well as I_{Kr} (hERG) leads to improved prediction of their clinical risk^{7,8}. In addition to $Na_v1.5$ expressed in a cell line, we also investigated hERG expressed in HEK cells using the Port-a-Patch or Port-a-Patch mini.

Application Note

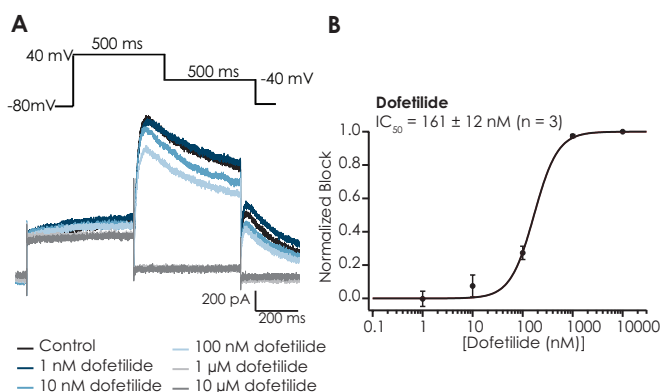


Figure 4: hERG current recorded from HEK cells using the Port-a-Patch mini. **A** Raw data traces elicited using a standard 2-step voltage protocol (shown at the top) and block by increasing concentrations of dofetilide. **B** Concentration response curve for an average of 3 cells. The data was fit with a Hill equation revealing an $IC_{50} = 161 \pm 12 \text{ nM}$.

The Port-a-Patch mini was used to record hERG expressed in HEK cells. The recordings were performed on the Port-a-Patch mini at room temperature using a standard 2-step voltage protocol. The external solution was exchanged manually using a pipette. In this way, low amounts of compound are required. Dofetilide blocked hERG expressed in HEK cells at room temperature with an IC_{50} of $161 \pm 12 \text{ nM}$ ($n = 3$), in good agreement with the range found in the literature^{2,7}.

In another set of experiments, the CiPA step-ramp voltage protocol was used to elicit hERG expressed in HEK cells at physiological temperature ($37 \pm 1^\circ\text{C}$) on the Port-a-Patch (voltage protocol is shown at the top of Figure 5A). In these experiments, the temperature controlled External Perfusion System for the Port-a-Patch was used to apply dofetilide. When the temperature of the bath solution was raised to physiological temperature in control solution, the peak amplitude increased approximately 5x compared to room temperature (RT) (Figure 5). The current was then blocked by $10 \mu\text{M}$ dofetilide.

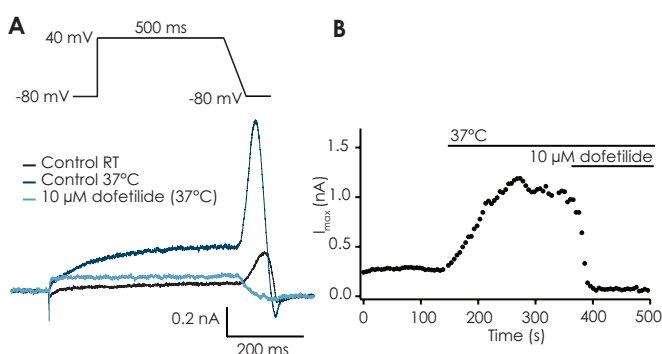


Figure 5: hERG current recorded from HEK cells using the Port-a-Patch and temperature controlled External Perfusion System. **A** Raw data traces elicited using a CiPA step-ramp voltage protocol (shown at the top) at RT, 37°C and following and block by $10 \mu\text{M}$ dofetilide. **B** Corresponding timecourse of the experiment. Increasing the temperature of the bath solution dramatically increased peak amplitude of hERG and the current was completely blocked by $10 \mu\text{M}$ dofetilide.

In conclusion, I_{Na} -Peak and I_{Na} -Late could be reliably recorded from hiPSC-CMs using the Port-a-Patch mini at room temperature. I_{Na} -Late which was activated by ATX-II was blocked by external application of ranolazine. Additionally, I_{Na} -Peak mediated by $Na_v1.5$ expressed in CHO cells was reliably recorded and blocked by tetracaine in a concentration-dependent manner. hERG expressed in HEK cells was recorded on the Port-a-Patch mini at RT and the current was blocked by increasing concentrations of dofetilide. The Port-a-Patch with temperature-controlled External Perfusion System was used to increase the temperature from RT to 37°C . This resulted in a large increase in the current measured during the ramp phase of the voltage protocol and the current was completely inhibited by a full block concentration of dofetilide. Using the Port-a-Patch or Port-a-Patch mini, different voltage protocols can be used, in these experiments we used a single voltage step protocol for $Na_v1.5$ recordings, along with a step-ramp protocol to enable both peak and late current to be recorded in one sweep. For hERG recordings we used a standard double voltage step protocol and a step-ramp protocol recommended by CiPA.

Application Note

Nanion offers planar patch clamp systems with varying levels of throughput and automation. The Port-a-Patch and Port-a-Patch mini are miniaturized patch clamp systems supporting giga-seal recordings from one cell at a time. The devices offer fast and easy access to high quality patch clamp data with only minimal training. Not only powerful research tools but also ideal for educational purposes and evaluation of cells and ion channels. The Patchliner and SyncroPatch 384 are fully automated robotic systems for recording from up to 8 or 384 wells simultaneously. All devices are compatible with recording ion channels important in safety screening as suggested by the CiPA initiative⁹. This is possible in both heterologous expression systems, e.g. HEK293 and CHO cells, and potential cardiac model cells such as stem cell-derived cardiomyocytes.

Nanion's products are used in all aspects of drug screening and safety testing due to their ease-of-use coupled with increased throughput or high throughput (HTS). The whole portfolio from the smallest patch clamp rig in the world, Port-a-Patch, to the automated patch clamp in-

strument with the highest throughput, SyncroPatch 384, are valuable for applications in target identification, efficacy, safety or HTS.

	Basic Research	Target Discovery	Target Validation	Assay Develop.	Drug Screening	Hit Validation	Lead Optimiz.	Safety / Toxicology
Port-a-Patch								
Patchliner								
SyncroPatch384								
CardioExcyte								
SURFER 96 SE								
SURFER N1								
Orbit 16 TC								
Orbit mini								

Figure 6: Accelerating Drug Discovery. Nanion's products are used in all aspects of drug screening, from basic research right through to lead optimization and safety testing.

References

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Methods

Cells

CHO-Nav1.5 recombinant cell line (CT6007) and HEK-

hERG (CT6001) were kindly supplied by Charles River, USA. Cardiosight-S[®] cells were kindly supplied by NEXEL.

Cell culture

Cells were cultured and harvested according to Nanion's standard cell culture protocols.

Electrophysiology

Whole cell patch clamp recordings were conducted according to Nanion's standard procedure for the Port-a-Patch and Port-a-Patch mini. Currents were recorded according to the CiPA protocol or according to standard protocols. Nav1.5 late currents were activated by application of 100 nM ATX-II in the external recording solution. Compounds were made as a 10 mM stock in DMSO and diluted in external recording solution to the concentrations indicated.