

Automated Tight Seal Electrophysiology for Assessing the Potential hERG Liability of Pharmaceutical Compounds

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Abstract: Unintended inhibition of the cardiac potassium channel human *ether-a-go-go*-related gene (hERG) is considered the main culprit in drug-induced arrhythmias known as *torsades de pointes*. Electrophysiology is the most reliable *in vitro* screening method for identifying potential cardiac hERG liabilities, but only the recent advent of planar electrode-based voltage clamp electrophysiology promises sufficient throughput to support the drug testing needs of most drug discovery programs. We have assessed the reliability of this new format of the voltage clamp technology in measuring the activity of small molecules on the hERG channel. Based on the results herein of a screening against a panel of well-characterized hERG-active and -inactive molecules, we demonstrate that planar electrode electrophysiology, utilizing the *Sealchip*TM and *PatchXpress*TM technology platform (AVIVA Biosciences Corp., San Diego, CA), is comparable to traditional electrophysiology based on glass micropipettes in its reliability and data content. The new technology will allow significantly higher throughput and more thorough testing of pharmaceutical compounds.

Introduction

DURING THE LAST DECADE, drug-induced *torsade de pointes* arrhythmias have been a major cause of serious adverse events, which has resulted in the withdrawal of many drugs from the market.^{1,2} These arrhythmias, which can lead to sudden death, result from a prolongation of the QT interval in the electrocardiogram, reflecting a prolonged cardiac action potential that may produce early afterdepolarizations. While the modulation of many cardiac ion channels could induce arrhythmias by action potential prolongation, the condition of drug-induced *torsade de pointes* has been highly correlated with the blockade of the K⁺ channel hERG (for reviews, see Keating and Sanguinetti³ and Roden⁴). The current carried by this

channel is activated during repolarization, and its magnitude is a key determinant of the cardiac action potential duration. It has been proposed that structural features of the hERG channel make it particularly prone to promiscuous blockade by small molecules.⁵⁻⁷

The pharmaceutical industry and regulatory agencies worldwide have become increasingly aware of the potential for new small molecule drugs to display hERG-blocking activity. Various approaches have recently been used to assess the hERG-blocking activity of compounds during drug discovery, from whole-animal electrocardiography studies to cardiac single-cell action potential prolongation studies, to determine drug safety prior to clinical testing. Because of the costs of drug discovery programs, the pharmaceutical industry strives to test for

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ABBREVIATIONS: CHL, Chinese hamster lung; CHO, Chinese hamster ovary; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethyl sulfoxide; D-PBS, Dulbecco's phosphate-buffered saline; FBS, fetal bovine serum; HEK, human embryonic kidney; hERG, human ERG (*ether-a-go-go*-related gene); HPLC, high-performance liquid chromatography; IC₅₀, 50% inhibitory concentrations; PBS, phosphate-buffered saline; R_a, access resistance; R_m, membrane resistance; R_e, electrode resistance; C_m, membrane capacitance.

drug liabilities earlier in the discovery process, but the number of compounds tested during this phase requires higher throughput methodologies. The most commonly used high-throughput methodologies to test compounds for activity on the hERG channel make use of mammalian cell lines expressing the hERG channel and involve one of the following approaches: binding assays, fluorescent voltage-sensitive dyes, or Rb^+ efflux (for a review, see Xu *et al.*⁸). These methods offer high throughput and low cost, but can only provide data of limited quality and reliability, making them prone to both false-negative and false-positive results. By far the most accurate and reliable technology for assessing the activity of compounds on hERG is voltage clamp electrophysiology. A recent study of 52 drugs² has provided evidence that *in vitro* electrophysiological assessment of compounds' activity on the cloned hERG channel is a reliable predictor of the cardiac liability *in vivo*. The International Committee for Harmonization is currently recommending a combination of *in vitro* hERG electrophysiology and *in vivo* QT measurement for new drug candidates (ICH S7B; see www.ich.org/MediaServer.jserv?@_ID=505&@_MODE=GLB).

Compounds designed to hit various targets and belonging to different structural scaffolds have been shown to have significant inhibitory activity on the hERG channel in the range of therapeutically relevant concentrations. The hERG activity of small molecules does not seem to closely track any specific scaffold, and within the same scaffold small differences in substituents can have a profound impact on the molecule's ability to block the hERG channel (see, for example, Woosley *et al.*⁹). As a result, hERG testing must be applied to a large number of compounds during the drug discovery process to ensure that hERG-active features are not built into molecules during lead optimization, and only electrophysiology can provide the quality of data necessary to optimize leads. This poses a challenge since traditionally electrophysiology is a labor-intensive, low-throughput method that is not efficient enough to support the synthesis capacity of typical medicinal chemistry projects. On average, the throughput of electrophysiology experiments is limited to just a few compounds per day, excluding it for screening a large number of molecules. In an effort to obviate these limitations, recently AVIVA Biosciences Corp. (San Diego, CA) and Axon Instruments (Union City, CA) have co-developed a technology that allows performing in parallel multiple automated electrophysiology experiments with a significant increase in throughput compared with the manual version. This new-generation electrophysiology instrument (PatchXpressTM) makes use of a planar chip (SealChipTM)¹⁰ with 16 independent chambers each containing a micro fabricated hole used to gain electrical access to the cells. The proprietary modification of the chip surface facilitates the

formation of tight ($G\Omega$) seals between the chip and the cells. In addition, the small volume of the chambers reduces the consumption of compounds in pharmacology experiments.

In the present work we report the data from the optimization and validation of the new technology applied to the determination of small molecules' activity on the hERG channel.

Materials and Methods

Cell preparation

CHO cells stably transfected with hERG cDNA and expressing hERG channels were derived at AVIVA Biosciences. Cells were cultured in T-75 (BD FalconTM, BD Biosciences, Bedford, MA) flasks using standard culture medium DMEM/F12 containing 10% FBS (Invitrogen, Carlsbad, CA), 1% penicillin with or without streptomycin, and 500 $\mu\text{g/ml}$ G418. Cells were propagated using trypsin (0.05%)/EDTA at a 1:5–1:40 ratio. CHO-hERG cells to be used on the PX7000A (Axon Instruments) were never more than 90% confluent at the time of isolation. Cells were harvested using Accumax (Innovative Cell Technologies, San Diego). Briefly, cells were washed twice in D-PBS (Invitrogen) without calcium and magnesium, and a 1:4 dilution of Accumax solution was added and incubated at room temperature for 2 min. Following removal of the first aliquot, a second aliquot of Accumax (1:4 dilution) was added to the cells and incubated for 4 min at 37°C. DMEM (10 ml) without calcium and containing 10% FBS was then added to quench Accumax digestion. Subsequently cells were centrifuged at 1,000 rpm for 2 min in a clinical centrifuge and resuspended in 5 ml of recovery media (calcium-free DMEM containing 10% FBS).

Electrophysiology

Whole-cell recordings were performed using the PX 7000A with AVIVA's SealChip technology. CHO-hERG cells were transferred from recovery media to the recording chambers containing external PBS (D-PBS). This solution contained (in mM): NaCl (138), Na_2HPO_4 (8.1), KCl (2.67), KH_2PO_4 (1.47), MgCl_2 (0.5), CaCl_2 (0.9), glucose (5.6), and sodium pyruvate (0.33). The intracellular recording solution contained (in mM): potassium glutamate (110), KCl (20), NaCl (8), MgCl_2 (1), HEPES (10), EGTA (10), and Mg-ATP (4). The pH was adjusted to 7.25 with KOH, and the final osmolarity was to ~ 285 mOsm.

Following addition of the cells to the recording chamber, positive pressure (6 mm Hg) was applied for 2 s, and subsequently negative pressure (-45 mm Hg) was applied to draw a cell onto the top of the electrode hole. A

repeating negative pressure ramp from 0 to -50 mm Hg was then utilized until seal resistance was greater than 1 G Ω . After achieving a Giga-seal, the pressure was ramped from -40 to -250 mm Hg repeatedly until the membrane patch within the aperture ruptured to achieve whole-cell configuration.

Cells were voltage clamped at a holding potential of -80 mV. An hERG measurement protocol was applied at 12-s intervals, consisting of a depolarizing prestep to -50 mV for 300 ms to serve as a baseline for current amplitude measurement, then activation of the hERG current by a step to $+20$ mV for 5 s, and then finally measurement of the peak of the deactivating current during a repolarizing step back to -50 mV for 5 s to remove the inactivation. The peak amplitude of the deactivating current was assumed to be equivalent to the steady-state activation current at $+20$ mV.

Compound handling and dilutions

All compounds were prepared as 10 mM DMSO stock in glass vials. Stock solutions were mixed by vigorous vortex-mixing and sonication for about 1 min at room temperature. All compounds were diluted for testing within 30 min before use. With the exceptions of the experiments reported in Fig. 2, in which compounds were diluted in polystyrene plates, all compounds were prepared in glass plates (Biotech Solutions, Mt. Laurel, NJ). Equal amounts of DMSO (0.1%) were present in all final dilutions.

Drug adsorption by HPLC

Adsorption of 1 μ M terfenadine to compound plates of different materials was studied using the HPLC analytical method. Compound plates of either polystyrene (Corning, Inc., Corning, NY) or glass (Biotech Solutions) were chosen. Both plates are in the 96-well format and have similar well volumes, resulting in a comparable surface-to-volume ratio. Fifty-microliter samples of 1 μ M terfenadine were deposited in the plates and collected after 15 min of incubation in each plate, and subsequently injected into the high-performance liquid chromatograph for adsorption analysis. As the sample was eluted from the column, a peak signal due to terfenadine was detected and recorded on the chromatogram. The area under the peak is then integrated and normalized to the control (1 μ M terfenadine). Each reading was done in triplicate.

PatchXpress procedures

The procedure designed to determine the IC_{50} value of the compound consists of a four or five concentration dose-response curve; the procedure to test percent block only is based on one or two concentrations. For both procedures, after achieving whole-cell configuration, the

cells were monitored for 90 s and washed with external solution for 66 s. The voltage protocol described above was then applied to the cells every 12 s and throughout the whole procedure. Only cells with above-threshold recording parameters ($R_m > 200$ M Ω ; $R_a < 10$ M Ω ; tail current > 150 pA and stable) were used for the drug dose-response procedure. The stability of the current was determined by measuring the difference between tail current peak amplitude of eight sweeps and the average of these sweeps; this difference should not exceed 0.2% for a cell used in the dose-response procedure.

D-PBS with 0.1% DMSO (vehicle) was applied to the cells to establish the baseline before compound testing. After allowing the current to stabilize for 3–5 min the test compounds were applied at increasing concentrations. Forty microliters of compound solution was added two or three times at 60-s intervals to each cell. When the compound's effect reached steady state, the next dose (or positive control) was added. Washout of compound was performed until the recovery of the current reached steady state. As a positive control 1 μ M cisapride was applied in all studies.

Data analysis

Data were analyzed using DataXpress, Clampfit (both by Axon Instruments), and Origin 7 (Originlab Corp., Northampton, MA). The baseline current (assigned as 100% block) was established by addition of 1 μ M cisapride. The reference current (0% block) was established by averaging of the last five sweeps of the 0.1% DMSO addition step.

IC_{50} values were estimated by fitting Hill equation to the data and recording the concentration that gave 50% inhibition. The coefficient of variation was calculated as (standard deviation/mean). The Z' factor was determined using the formula:

$$Z' = 1 - (3\sigma_{c+} + 3\sigma_{c-})/|\mu_{c+} - \mu_{c-}|$$

where σ_{c+} and σ_{c-} are standard deviations for cells treated with 1 μ M cisapride and 0.1% DMSO, respectively, and μ_{c+} and μ_{c-} are the corresponding means.

Materials

Cisapride and verapamil were obtained from Tocris (Avonmouth, UK). Amiodarone, terfenadine, pimozone, haloperidol, astemizole, thioridaxine, quinidine, aspirin, and diphenhydramine were obtained from Sigma (St. Louis, MO). Plastic (polystyrene) 96-well assay plates (Costar 3896) were from Corning. Glass vials used as inserts in plates for compound dilution and storage before applying to the cells were from Biotech Solutions (catalog number 4050FB-917).

TABLE 1. RECORDING PARAMETERS

	Mean \pm SEM (n = 102)
R_c (M Ω)	2.25 \pm 0.02
R_a (M Ω)	8.78 \pm 0.50
R_m (M Ω)	991 \pm 48
Current amplitude (pA)	340 \pm 14
Cell capacitance (pF)	10.6 \pm 0.40
Current density (pA/pF)	32 \pm 1

Results

In the present work we assessed the reliability of planar voltage clamp electrophysiology for estimating the activity of hERG potassium channel blockers. We also report the results of our assay optimization effort for the SealChip and PatchXpress platform. Voltage clamp electrophysiology¹¹ is considered the most reliable and accurate method to measure ion channel currents and their modulation by pharmacological compounds. The PatchXpress can provide high-fidelity whole-cell electrical access and allows voltage clamp recording. In PatchXpress the recording electrode consists of AVIVA's SealChip planar electrode, which contains 16 independent recording chambers each having a 1–2- μ m hole at the bottom. The seal is established from the bottom of the chamber. With the SealChip electrode, compounds are delivered from a 96-well plate into the recording chambers by means of disposable tips.

At the beginning of the experiment dissociated cells are dispensed in suspension in each chamber, and a single random cell landing on the hole at the bottom of the chamber can form a tight (G Ω) seal with the surface of the SealChip. Suction applied through the hole can then rupture the patch of membrane and establish electrical continuity between the inside of the cell and the underlying electrode chamber. The cell is then monitored for a brief period of time to assess its stability, and subsequently recording is started by measuring hERG baseline current (which serves as the predrug control current) to

determine the stability and quality of the ionic current. Cells with high-quality, stable-recording configuration and exhibiting a stable baseline are then used to determine the effects of the test compounds. The parameters describing the recording configuration and data quality from over 100 experiments are reported in Table 1. The voltage clamp quality parameters (membrane capacitance C_m , R_m , and R_a) were measured before the application of drugs, and were used by the automation software as criteria for determining whether to perform or terminate the experiment. Steady-state current was measured in response to a 20 mV activation step before the application of drugs. Under these conditions using our CHO-hERG cell line following a 5-s depolarizing step to +20 mV we measured an average tail current amplitude of 340 \pm 14 pA when the voltage was stepped back to -50 mV ($n = 102$). The voltage dependence of activation was measured in a subset of experiments by stepping to different voltages from a holding potential of -90 mV (Fig. 1A). The measured voltage dependence of was $V_{1/2} = -13.5 \pm 3$ mV ($n = 8$) (Fig. 1B).

In order to determine the sensitivity and accuracy of the measurement performed using the PatchXpress, we have used a panel of known hERG blockers (amiodarone, terfenadine, pimozone, astemizole, thioridazine, haloperidol, bepridil, cisapride, quinidine, and verapamil) for which extensive literature data are available.² These compounds span a wide range of therapeutic indications, belong to different structural families and possess different grades of hydrophobicity, ranging in cLogP values from 2.79 to 8.95. We have also included in the study two compounds known to lack significant hERG activity (diphenhydramine and aspirin). One example of the measurements we have performed is shown in Fig. 2, and the summary of the testing results is reported in Table 2 where the IC₅₀ values obtained using the PatchXpress are compared with those reported in a recent literature survey.² The PatchXpress data we have obtained show a good correlation with the lower end of the data range published in the literature ($r^2 = 0.73$) (Fig. 3). Amiodarone appears to be an outlier (Fig. 3, arrow), and when

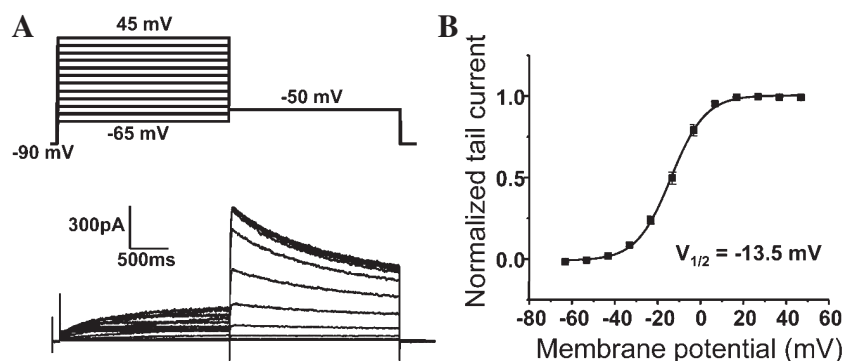


FIG. 1. Voltage dependence of activation. (A) Voltage dependence of activation was determined by measuring the peak tail current at -50 mV following activation steps at different voltage potentials between -65 and +45 mV (10 mV intervals): (top panel) stimulation waveforms used and (bottom panel) sample traces recorded in response to the stimulation protocol. (B) The data from eight cells stimulated as described in (A) were pooled, and the Boltzmann equation was fit to the normalized amplitude of the peak tail current.

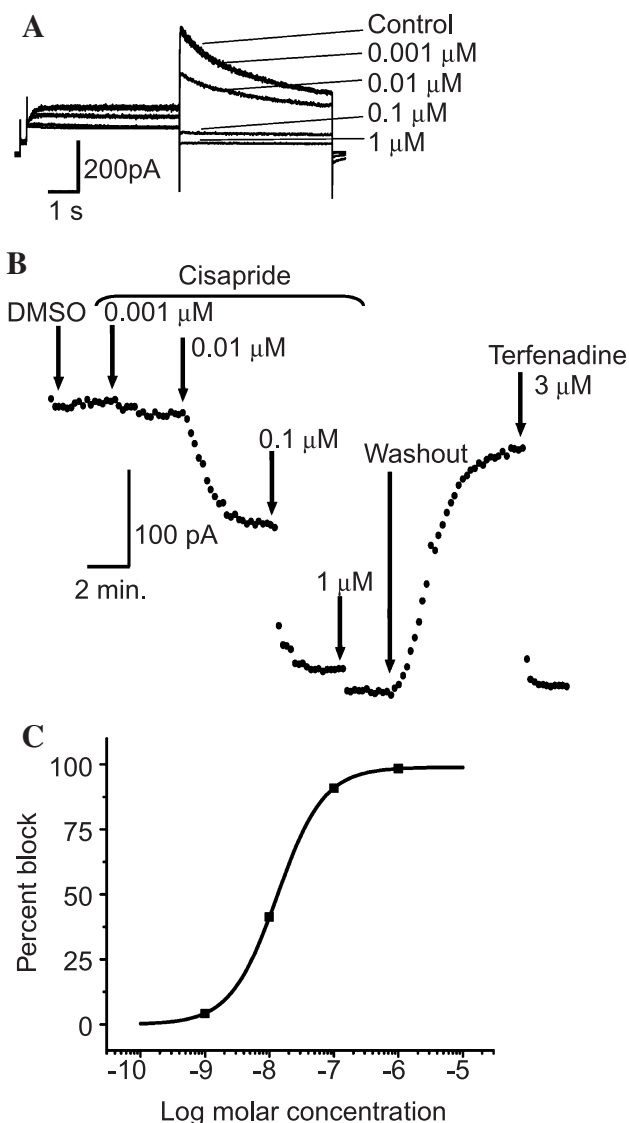


FIG. 2. Measurement of the hERG-inhibitory activity of cisapride. **(A)** Example traces of hERG currents recorded in the presence of increasing concentrations of cisapride. Cells were voltage-clamped at a holding potential of -80 mV, and hERG current was activated by depolarizing steps, first to -50 mV for 300 ms, then to $+20$ mV for 5 s to activate the channels, and finally back to -50 mV for 5 s. Cisapride concentrations are indicated on the side. **(B)** Time course of the hERG channel inhibition achieved by cisapride for the data shown in **(A)**. Each dot represents the peak tail current amplitude measured in response to the stimulation protocol described in **(A)**. Cells were stimulated every 12 s. Vertical arrows indicate the time of application of 0.1% DMSO (negative control), or increasing concentrations of cisapride. Cells were washed with D-PBS. Terfenadine ($3 \mu\text{M}$) was used at the end of the experiment as a positive control. **(C)** The Hill equation was fit to the data from the experiment in **(A)** and **(B)**.

it was excluded from the linear fitting, we obtained $r^2 = 0.87$ (see Discussion).

During the course of the study we have identified several parameters that can affect the throughput of automated electrophysiology and/or the reliability of the data:

Drug	<i>cLogP</i>	<i>IC</i> ₅₀ (nM)	
		Conventional ^a	AVIVA ^b
Amiodarone	8.95	1,000–9,800	136 ± 10
Terfenadine	6.5	20–200	44 ± 3
Pimozide	6.4	15–55	8 ± 0.7
Bepiridil	6.33	600–13,000	124 ± 26
Astemizole	5.65	1–26	6 ± 0.3
Thioridazine	5.13	33–1,250	246 ± 12
Haloperidol	3.98	27	30 ± 2
Cisapride	2.97	2–45	13 ± 1
Quinidine	2.79	300–1,000	989 ± 72
Verapamil	4.47	140–800	391 ± 20
Diphenhydramine	3.27	30,000	6,800 ± 530
Aspirin	1.3	>30,000	>30,000

^aData from Redfern *et al.*²

^bData from present study from three to 17 cells per measurement.

- cell harvesting protocol
- cell line background
- compound plate material
- protocol of compounds addition to the cells

Cell harvesting protocol

In our experience the condition of the cells at the beginning of the experiment is perhaps one of the most crit-

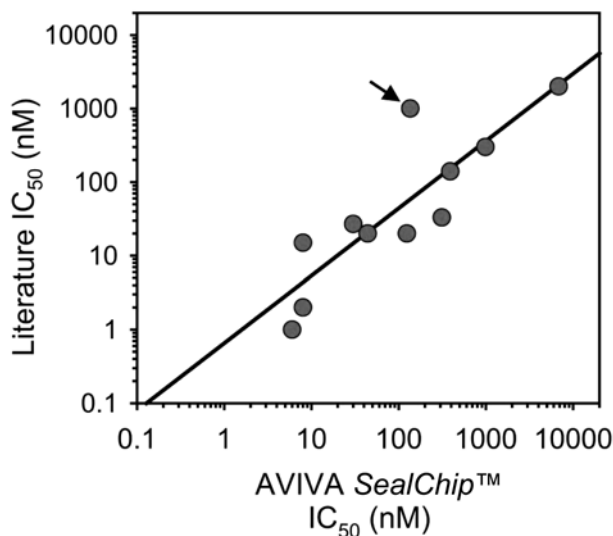


FIG. 3. Correlation between conventional and automated electrophysiology. For the set of compounds listed in Table 2, the *IC*₅₀ determined in this study using *SealChip* and *PatchXpress* were plotted against the *IC*₅₀ obtained with traditional patch clamping and reported in the literature. For the compounds for which a range of values was described in the literature, only the value at the low end of the spectrum was used in the correlation plot. The arrow indicates amiodarone.

ical factors determining both the seal success rate as well as the overall quality of the data. During cell isolation from the culture flasks, under- or overdigestion with proteolytic enzymes can have a deleterious effect on the assay (data not shown). Additional important parameters of the isolation are: the density of cell seeding, the duration of culture before harvest, and the recovery time after harvesting. Figure 4 shows the seal success rate (on the *y*-axis) for cells harvested 16 h ($n = 48$ cells per time point) or 36 h ($n = 48$ cells per time point) after seeding, and allowed to recover for 0 to 60 min (on the *x*-axis). For cells cultured for 16 h, immediately after the enzymatic isolation treatment and cell wash the seal success rate is relatively low (<40%). Between 15 and 60 min after the end of the harvesting procedure the seal success rate is between 60% and 70% (Fig. 4A). Cell processed according to this protocol exhibit, however, relatively small currents, and the current density is on average 18 ± 2 pA/pF ($n = 80$) (Fig. 4C). Cells cultured for 36 h before harvesting have a different profile (Fig. 4B). The seal success rate consistently exhibit a dip at 15 min after the isolation and recovers within 1 h. Cells cultured for 36 h before harvesting have a current density of 25 ± 2 pA/pF ($n = 116$) (Fig. 4D).

While the current amplitude does not vary in a significant way between cells cultured 16 or 36 h, we have noticed that the fraction of cells expressing detectable currents is higher for cells cultured 36 h prior to harvesting (cells with detectable hERG at 36 h, $84 \pm 6\%$, $n = 138$; cells with detectable hERG at 16 h, $57 \pm 8\%$, $n = 140$; $p < 0.05$, Student's *t* test). We routinely use cells cultured for 36 h and allow the cells to recover for 1–3 h before recording.

We have also compared the propensity of different cell lines to form GΩ seals on the *SealChip*. Four commonly used cell lines—CHO, HEK, CHL, and LTK—were harvested using either trypsin or Accumax and tested 1 h later for seal formation on *SealChip*. Figure 5 shows that CHO and LTK are the most efficient cell lines at forming tight seals on the *SealChip*, regardless of the harvesting protocol used. It is conceivable that this result is due to differences in the composition of the phospholipid bilayer, the polysaccharides, or the membrane proteins expressed on the surface of the various cell lines. We have not further investigated the source of the observed differences. It should also be noted that the cell lines used for the experiments in Fig. 5 did not overexpress any exogenous ion channel. In principle, stable transfection to induce overexpression of hERG or other ion channels could alter the sealing propensity of the cell lines.

Compound plate material

In traditional patch clamp electrophysiology the delivery of compounds to the cell being tested is typically

achieved using continuous superfusion from a reservoir constituted by a syringe or a polypropylene tube. In the PatchXpress assay, compounds are delivered by individual delivery steps in which disposable pipette tips are used to transfer the compounds at their final concentration from 96-well plates. In the PatchXpress, the absence of continuous superfusion of the compounds could result in errors in the effective concentration in solution due to adsorption to the surface of the compound plate, the surface of the disposable plastic tips, or the surface of the *SealChip*, or into other cells that are not voltage clamped but are contained within the chamber. We have compared the hERG block measured for four compounds (300 nM amiodarone, 50 nM terfenadine, 10 nM cisapride, and 1 μM quinidine) using compound plates made with polystyrene or glass. Figure 6 shows the shift in hERG inhibition observed when the same set of compounds were prepared and stored for 15 min in a polystyrene plate or in a glass plate. The reduction in apparent inhibition associated with the use of the polystyrene plate is more pronounced for the compounds with higher cLogP values (amiodarone, cLogP = 8.95; terfenadine, cLogP = 6.5). This suggests that the more hydrophobic compounds may be adsorbed to the polystyrene compound plate. We have used HPLC to quantify the differential adsorption of terfenadine (1 μM) to polystyrene and glass (see Materials and Methods). Following a 15-min incubation in the two type of plates, $9 \pm 0.4\%$ ($n = 3$) of the starting terfenadine was adsorbed on the glass plate, while $26 \pm 2\%$ ($n = 3$) was adsorbed on the polystyrene plate ($P < 0.05$, Student's *t* test).

The use of different tip brands or of different *SealChip* surface modifications did not have a measurable effect on the percent block achieved by hERG inhibitors (data not shown).

Compound addition protocol

Given the possibility that the compounds' effective concentration may be reduced through adsorption to the compound plate, we decided to minimize this effect by exclusively using glass plates, as discussed above. We have also explored the effect of a compound addition procedure that involves repeated application of the same drug-containing solution. Figure 7 shows the comparison of single, double, or triple compound additions for terfenadine, amiodarone, cisapride, and quinidine (same concentrations as in Fig. 6). The triple addition protocol had a significant effect for the two most hydrophobic compounds tested (amiodarone and terfenadine), but there was no increased inhibition going from single to triple addition for the more hydrophilic compounds cisapride and quinidine. The effect of multiple additions is more evident when the

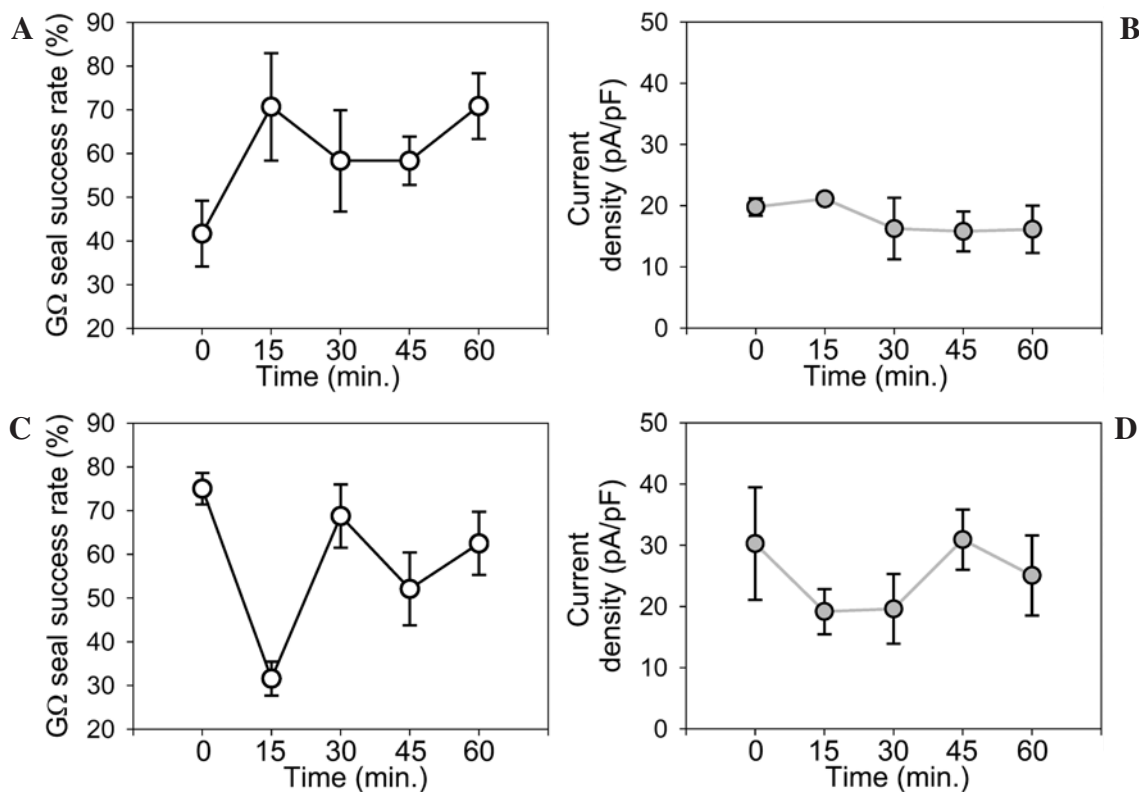


FIG. 4. Effect of cell recovery on seal success rate and hERG current density. (A) Cells were trypsinized and seeded into T-75 culture flasks and cultured for 16 h (overnight). Harvesting was performed as described in Materials and Methods. At the times indicated after the end of the harvesting procedure the cells were dispensed in *SealChip* chambers on the PatchXpress and tested for GΩ seal formation. (B) For the same cells tested in (A), the current density was determined right after breaking in and achieving whole-cell configuration. (C) Seal success rate was determined as in (A) but the cells were cultured for 36 h (over 2 nights) before harvesting. (D) Current density for the cells in (C) as measured immediately after achieving whole-cell configuration.

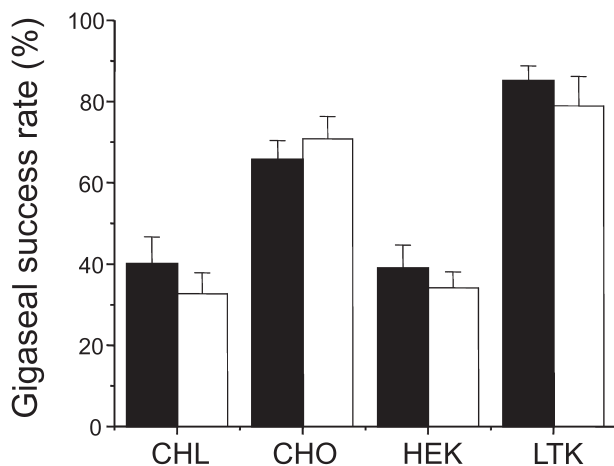


FIG. 5. Propensity of different cell lines to form GΩ seals on the *SealChip*. Indicated cell lines were harvested using trypsin (■) or Accumax (□). The duration of enzymatic digestion was adjusted to result in ~95% of cells being round and detached in all conditions for all the cell lines. At 1–2 h after harvesting the cells were tested in PatchXpress for their ability to form seals $\geq 1\text{G}\Omega$ ($n = 3$ for each condition).

different addition steps are spaced by at least 1 min. In our hands multiple additions delivered at shorter time intervals are similar to single addition.

Reliability and robustness of the assay

To determine the reliability of the assay we have repeated single concentration determination over the course of several days and determined the coefficient of variation of the inhibition. A partially blocking concentration of quinidine ($1\ \mu\text{M}$; $\sim\text{IC}_{50}$) was used for measurements of hERG inhibition over the course of 8 days. The measured coefficient of variation over an 8-day period was 12% ($n = 30$). Cumulative data analysis was used to determine the Z' factor for the PatchXpress hERG assay. For determination of the Z' factor the hERG tail current from 74 cells was measured in D-PBS and in the presence of 0.1% DMSO (control) and $1\ \mu\text{M}$ cisapride (3-min incubation, test condition). The measured Z' was 0.88 ($n = 74$) (Fig. 8).

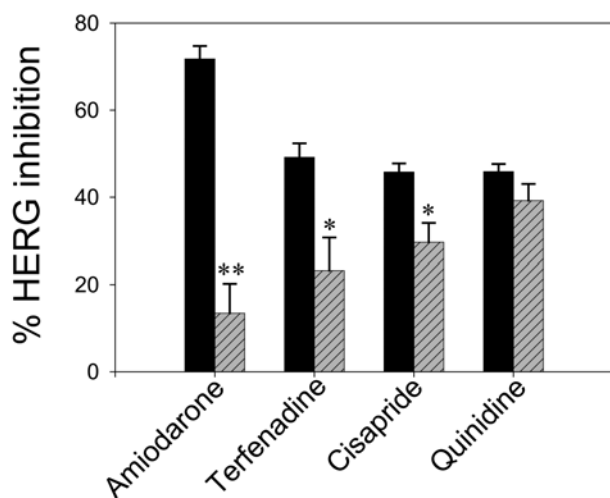


FIG. 6. Effect of the test compound's plate material on the measured hERG inhibition. Amiodarone (300 nM), terfenadine (50 nM), cisapride (10 nM), and quinidine (1 μ M), were prepared in D-PBS containing 0.1% DMSO. Final dilutions of the compounds were dispensed in either glass vials (■) or polystyrene 96 well plates (▨). After incubation for 15 min at room temperature the compounds were applied to cells. The inhibition of the peak tail current for the hERG channel is shown. * $p < 0.05$, ** $p < 0.01$, analysis of variance ($n = 3$).

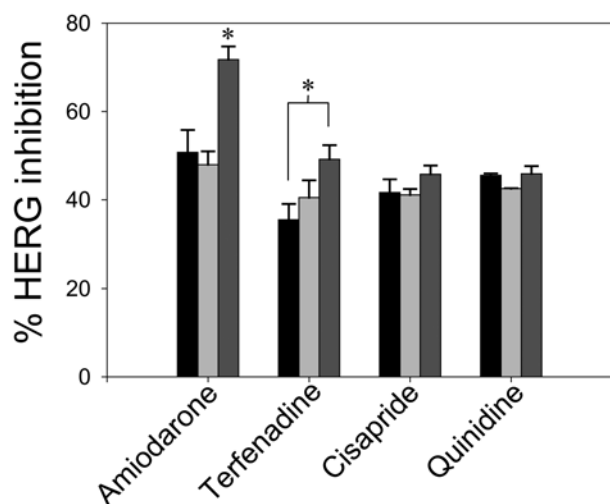


FIG. 7. Effect of the test compound's addition protocol on the measured inhibition of hERG current. Cells with above-threshold recording parameters (see Materials and Methods) were used in the compound testing procedure. The indicated compounds (same concentrations used in Fig. 6) were applied in a single step (solid bars) until the effect on hERG peak tail current reached steady state. In a parallel series of experiments the first compound's application was repeated a second time (light-gray bars). In the last series the compounds were applied a total of 3 times (dark-gray bars). * $p < 0.05$, analysis of variance ($n = 3-4$).

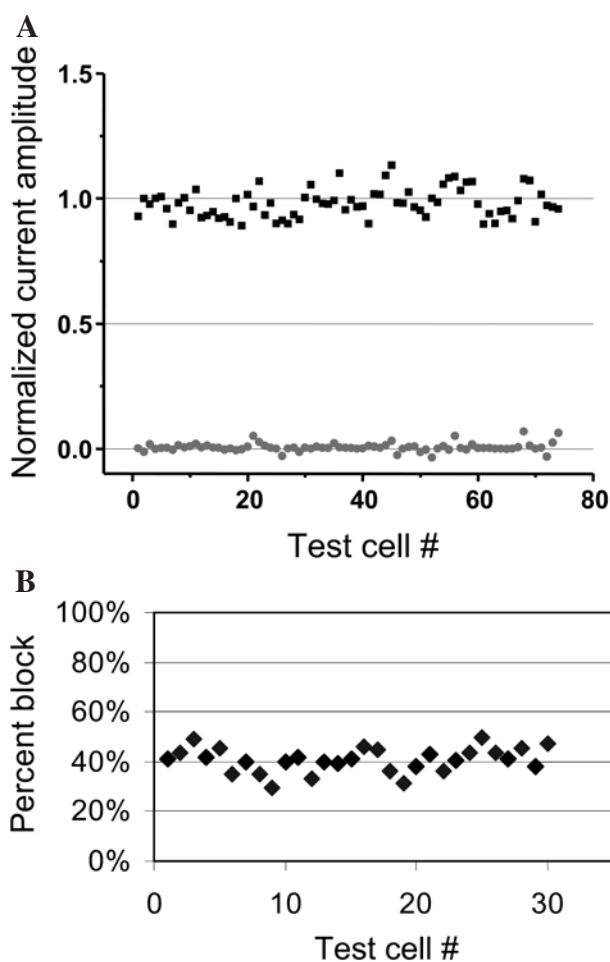


FIG. 8. Assay stability over time and Z' factor measurement. (A) The effect of 1 μ M quinidine on the hERG peak tail current was assessed in PatchXpress over several days following the recording and compound's application procedures described in Materials and Methods. (B) Z' factor (assay window) was determined measuring the peak tail current amplitude in D-PBS with or without 0.1% DMSO and 1 μ M cisapride ($n = 74$).

Discussion

In drug discovery projects, the most reliable *in vitro* approach for early assessment of potential cardiac liabilities of compounds is voltage clamp electrophysiology. The inhibition of the potassium channel hERG has been implicated in the occurrence of drug-induced arrhythmias. Voltage clamp electrophysiology allows the direct measurement of the hERG channel activity and the effects of inhibitory compounds. Traditional electrophysiology is a very-low-throughput technique. The net result is that very frequently the number of new molecules that can be generated by a given drug discovery program per unit of time far exceeds the number of molecules that can be tested for biological activity using electrophysiology. PatchXpress represents a significant advancement in

electrophysiology by allowing increased throughput without compromising the quality of the data. Using *SealChip* and *PatchXpress* technology a single operator can comfortably achieve a productivity of 70–100 data points per day, which is significantly higher than the 10–20 data points per day of conventional electrophysiology. We have tested the performance of AVIVA's planar electrode *SealChip* and the *PatchXpress* instrument in measuring the activity of compounds on the hERG channel. We have compared the results of our measurements with the data obtained using the traditional voltage clamp method. It should be pointed out that for many of the compounds discussed in this study the data reported in the literature show significant variability. This is not due to intrinsic limitations of traditional voltage clamp, but most likely originates from the fact that the hERG IC_{50} data reported by Redfern *et al.*² were measured in different laboratories, utilizing somewhat different experimental conditions. For example, it is well known that hERG channel activity and its pharmacological properties are affected by temperature. In the data sample reported by Redfern *et al.*,² not all measurements were performed at the same temperature, which could account for some of the variability. In addition, composition of extracellular or intracellular buffers and voltage protocol are additional parameters not controlled in the reference data sample by Redfern *et al.*,² which could contribute to the variability in the reported IC_{50} . These limitations of the reference data set we used for comparison could account for some differences between the values reported from traditional voltage clamp and our *PatchXpress* data. Overall, we observe a remarkable correlation between our data and the reference literature data.

In vitro voltage clamp hERG assay is used to predict the potential for cardiac toxicity of new compounds. So, another way to assess the quality and reliability of the hERG data obtained using *SealChip* and the *PatchXpress* is to evaluate, retrospectively, how predictive this new technology is of the clinical liabilities of compounds. For this type of analysis we have used the data and criteria described by Redfern *et al.*² Based on their studies of clinical compounds with well-documented cardiac toxicity, these authors have concluded that a good predictor of cardiac liability is obtained when the ratio of hERG IC_{50} over the effective unbound plasma concentration is below 30. We have compared our hERG IC_{50} values as measured in *PatchXpress* with the therapeutic plasma concentrations (unbound) and asked if, by applying the criteria by Redfern *et al.*,² the drug would be predicted to be safe. We have found that based on our IC_{50} measurements, seven of the tested drugs (pimozide, astemizole, thioridazine, bepridil, haloperidol, cisapride, and quinidine) are correctly predicted to have potential for QT prolongation and drug-induced arrhythmias (Fig. 9). By the criteria of Redfern *et al.*² the QT prolongation po-

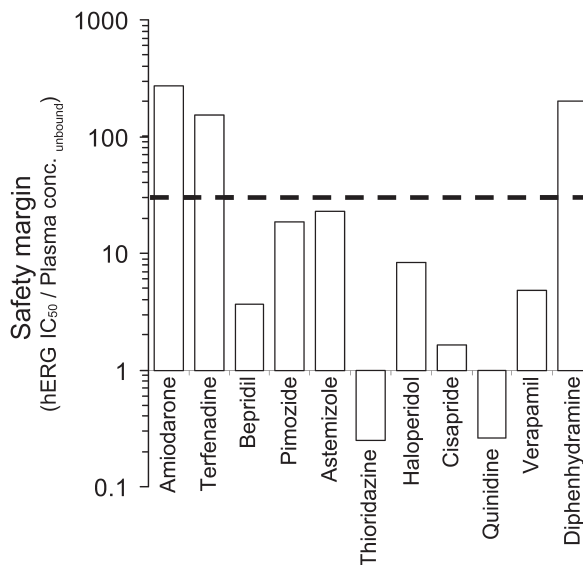


FIG. 9. Correlation between *Sealchip*–*PatchXpress* data and cardiac liabilities. Predicted safety margin for the compounds used in this study was calculated using the IC_{50} determined in this study (Table 2) and the lower end of the unbound plasma concentration for each compound reported by Redfern *et al.*² The dashed line highlights the threshold safety margin of 30-fold.

tential of pimozide would be missed based on the IC_{50} reported in the literature ($IC_{50} = 15–55$ nM; pimozide free plasma concentration = 0.43 nM, thus 30-fold safety margin at 12.5 nM). However, pimozide would be predicted to be unsafe based on the *PatchXpress* data we have measured. For amiodarone the criteria of Redfern *et al.*² would require an IC_{50} of 15 nM or lower in order to predict hERG-related toxicity. In this case both our *PatchXpress* value (136 nM) as well as the traditional patch clamping measurements reported in the literature (≥ 1 μ M) would have missed the potential for cardiac toxicity, even though the *PatchXpress*-based assay appears to be closer to the correct estimate than traditional patch clamping. In the case of terfenadine, the threshold for predicting toxicity (8.7 nM) is below the values measured in *PatchXpress* and the values reported in the survey by Redfern *et al.*² It should be pointed out, however, that more recent measurements¹² in traditional voltage clamp (9.4 ± 2.2 nM) come very close to the threshold for predicting toxicity.

Both traditional and *PatchXpress*-based electrophysiology may underestimate the hERG blocking activity of terfenadine and amiodarone. These compounds are highly hydrophobic with cLogP values of 6.5 and 8.95, respectively. It is conceivable that the hydrophobic nature of the molecules may cause the underestimate of the compound's potency, due to adsorption to the compound's plate, the delivery tip, and/or the *SealChip*. Alternatively terfenadine and amiodarone may indeed have

limited potency for hERG inhibition, as measured in electrophysiology, but by virtue of their lipophilic nature they may accumulate in the cell membrane or inside the myocardial cells and achieve significant hERG block even at very low plasma concentrations.

The PatchXpress data would also correctly predict the lack of cardiac toxicity of diphenhydramine. In the case of verapamil there is good concordance between the data from traditional voltage clamp and PatchXpress data. The predicted toxicity of verapamil is not an error due to intrinsic limitations of the PatchXpress technology but is due the lack of selectivity of this drug, which has Na⁺ and Ca²⁺ channel-blocking activities that potentially offset the hERG inhibition.

For the set of compounds used in this study traditional electrophysiology data, PatchXpress data, and *in vivo* data are available, and the following conclusions can be drawn:

1. Regardless of the specific methodology used (manual electrophysiology or PatchXpress), electrophysiology on the hERG channel is a good predictor of cardiac liabilities of compounds.
2. PatchXpress is equivalent to traditional patch clamping when it comes to accurately predicting a compound's toxicity.

Our results also indicate that when evaluating the potency of hydrophobic molecules special care should be used in choosing the material of the compound's plate and the addition protocol. Utilizing glass plates and multiple addition protocol can reduce artifacts due to compound adsorption.

We have also evaluated different cell line backgrounds for their efficiency in forming tight (GΩ) seals on the SealChip. In the absence of hERG overexpression, it appears that the CHO and LTK cell lines are probably the most indicated background for electrophysiology applications on the SealChip and PatchXpress platform. However, when choosing the background cell line the expression of endogenous channels, which differ in the various cell lines and which may interfere with the measurements, should also be taken into account.

The quality and reliability of the data generated by the SealChip and PatchXpress platform are comparable to those offered by traditional electrophysiology. The throughput, however, is significantly higher in PatchXpress and will allow the generation of data with high

efficiency and at a rate sufficient to support most medicinal chemistry programs.

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