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How *in vitro* influences *in silico* utilized for the prediction of *in vivo* — pilot study of the drug-induced pro-arrhythmic potency prediction

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Abstract: The current drug cardiac safety risk assessment paradigm is about to be changed. The discussed modifications cover clinical as well as pre-clinical sides. As for the latter, the pre-clinical assessment, it is planned to be based on the analysis of the drug-triggered multiple ion currents inhibition. Considering the variability in the *in vitro* patch clamp studies results, it would be of benefit to assess how these apparatus- and protocol-dependent differences influence the risk prediction and, eventually, the decision making. Four compounds, namely dextromethorphan, ketoconazole, terfenadine, and quinidine were screened for hERG inhibition with an automated patch clamp apparatus (CytoPatch™2). The results were then compared against the literature published data, and after being complemented with information about other current inhibitions and effective therapeutic plasma concentration, utilized for the *in silico* based safety assessment. Two endpoints were used: (1) the concentration dependent potential to induce early afterdepolarizations in the simulated action potential and (2) the arrhythmia-like disruption in the simulated pseudo-ECG signals. Data analysis results prove that IC₅₀ values, describing the inhibition potential, significantly differ among studies, and the choice of input data can greatly influence the *in silico* based safety assessment and thus the decision making process.

Key words: hERG, ionic channels, Patch Clamp, proarrhythmia.

Introduction

A lot has been said and written recently about the drug induced pro-arrhythmic risk screening. The main reason for the activity is the currently proposed drug pro-arrhythmic risk assessment modification [1, 2]. The intended changes cover all the main areas, beginning from the pre-clinical up to the clinical level. Proposed changes in the latter include the so-called thorough QT trials replacement with careful electrocardiography (ECG) evaluation in First-in-Human (FIH) studies using Exposure Response (ER) analysis in the evaluation of a drug's effect on cardiac repolarization [3]. Despite criticism [4], first results prove that QT assessment, performed in early phase studies with the use of an intense ECG schedule and ER analysis, can detect a small QT effect with the same confidence as a thorough QT study [5, 6].

Comprehensive safety assessment of drugs requires examining the multiple intrinsic and extrinsic factors what influence cardiac rhythms. The generation of action potentials in cardiac cells requires coordination of multiple currents that are mediated by sodium, calcium, and potassium ion channels. Drug-triggered modification of any of these currents or their combination can either be pro- or anti-arrhythmic. Therefore, at the pre-clinical level we cannot afford improper *in vitro* measurement. Proper assessment will be even more important with the MICE (multiple ion channel effects) initiative protocols introduced into the daily safety labs routine. Multiple channels multiplied by hundreds or thousands of compounds' results in a substantial number of data points.

These have to be generated, and what's even more important, generated with a high quality assuring proper decision making [7, 8]. It is likely that such decisions will, at least partially, be made based on the basis of *in silico* simulations. The focus of these simulations will be the drugs pro-arrhythmic potency assessment [9].

These days automated patch clamp devices are believed to give an optimal trade-off between accuracy and economic considerations. They offer medium-to-high throughput. Yet it should be clearly said that according to the well-known and often repeated expression "rubbish in, rubbish out" the *in vitro* data have to be of the highest possible quality, as even the best model will fail when improper data are provided. Often neglected factors (like temperature, composition of bath solution, and voltage protocol) can significantly influence the results and therefore modify model output.

Aims of the study

The ultimate aim of the cardiac-safety electrophysiological studies is to evaluate the pro-arrhythmic risk of a given compound based on the *in vitro* data, coming from experiments performed in various conditions which were used to feed the *in silico* model of a human cardiac myocyte. During this study we intended to evaluate the usability of

the novel automated patch clamp method and apparatus (CytoPatch™2) by testing our measurement results against previously published data on drug-induced ionic currents inhibition.

Materials and Methods

Drugs selection

The compounds were chosen following several criteria. Firstly, we wished to include drugs from various risk categories, based on the CredibleMeds classification [10]. This classification involves three main categories: known (*Substantial evidence supports the conclusion that these drugs prolong the QT interval AND are clearly associated with a risk of TdP, even when taken as directed in official labeling*), conditional/possible (*Substantial evidence supports the conclusion that these drugs can cause QT prolongation BUT there is either insufficient evidence at this time that these drugs, when used as directed in official labeling, are associated with a risk of causing TdP or risk appears only under certain conditions like excessive dose, hypokalemia, congenital long QT or by causing a drug-drug interaction that results in excessive QT interval prolongation*) Torsade de pointes (TdP) risk and not listed (assumed as safe, with no arrhythmia reports). We looked at the availability of electrophysiological *in vitro* data describing other, apart from IKr, potentially blocked currents. We also looked at the information about the pharmacokinetics, mainly the effective therapeutic plasma concentration (ETPC) and the protein binding (the so called fraction unbound). The final list of selected compounds with their brief characterization is given in Table 1.

Table 1. Compounds selected for the in vitro and in silico study.

Compound	CredibleMeds classification	Comments	Ref.
Dextromethorphan (DXM)	Not listed (considered as safe)	Considered as safe despite of relatively potent IKr inhibition. Metabolized by 2D6, plasma concentration and clinical effect will be CYP genetic polymorphic form dependent.	[22, 23]
Ketoconazole	Conditional Risk of TdP	QT prolongation reported yet lack of known TdP cases during monotherapy.	[24]
Terfenadine	Known Risk of TdP	Known as torsadogenic agent yet different mechanism as compared to quinidine. Considered as safe in physiological concentration, pro-arrhythmic potency significantly increases after CYP 3A4 inhibition (e.g. ketoconazole co-administration).	[24, 25]
Quinidine	Known Risk of TdP	Known as torsadogenic agent. Clearly pro-arrhythmic due to the mechanism of action.	[26]

In vitro study

Cell Culture. Stably transfected HEK 293 cells expressing the hERG ion channel (frozen Instant Cells, German Patent 10 2007 010 843.7-09, Cytocentrics Bioscience GmbH, Rostock, DE) were stored in liquid nitrogen. Each aliquot consisted of around 10 million cells. For use, the cells were thawed in extracellular buffer (EC) at room temperature (RT), centrifuged and re-suspended in EC. The cells were then moved to Cytocentrics Cell Reservoir, and used within 4 h.

Solutions and Drugs. The EC used for automated patch clamp recordings, thawing and resuspending of the HEK 293 cells, was composed of the following ingredients (in mM): 140 NaCl, 2.5 KCl, 2 MgCl₂, 2 CaCl₂, 10 HEPES, 10 glucose and 15 sucrose. pH was adjusted to 7.4 ± 0.1 (NaOH); the osmolality was 320 ± 5 mOsm/kg. The buffer was stored at 4°C, degassed and pre-warmed to RT prior to use. The intracellular buffer (IC) used for automated patch clamp recordings was provided by Cytocentrics Bioscience GmbH, Rostock, DE. It was composed of the following ingredients (in mM): 100 KGluconat, 20 KCl, 1 CaCl₂, 1 MgCl₂, 10 HEPES, 11 EGTA-KOH, 4 MgATP, 3 phosphocreatine-Na₂-H₂O, and 9 sucrose. pH was 7.2 ± 0.1 ; the osmolality was 295 ± 5 mOsm/kg. Aliquots were stored at -20°C, thawed prior to use, and used for maximum 4 h. All drugs were purchased from Tocris Biosciences. Stock solutions were prepared in DMSO for all compounds in concentrations as follows: quinidine 100 mM, ketoconazole 50 mM, dextromethorphan (DXM) 50 mM and terfenadine 100 mM. Aliquots of these stock solutions were stored in -20°C. For electrophysiological recordings working concentrations of the drugs were freshly prepared by diluting to respective millimolar concentrations in DMSO and then to micromolar concentrations in EC. Obtained working solutions were used for no more than 4 hours. Final concentration of DMSO did not exceed 0.1%.

Electrophysiological recording. Experiments were performed on automated patch-clamp device CytoPatch™2 (Cytocentrics Bioscience GmbH, Rostock, DE), according to a protocol described in [11]. In short, silicon dioxide microfluidic chips were used with two embedded quartz pipette tips (2 mm in diameter), each surrounded by a Cytocentering channel for capture and coordination of cells out of the cell suspension and to the pipettes. This setup allows for simultaneous seal formation and recordings in two cells. The sequence to whole-cell configuration (i.e. chip filling, cell capture, gigaseal formation, and patch rupture) was fully automated.

hERG outward tail currents were measured by executing the following pulse protocol every 10 s: from a holding potential of -70 mV, cells were voltage-clamped for 100 ms to -50 mV and then for 2 s to +40 mV. To evoke outward tail currents, a 2 s step to -50 mV followed (see Fig. 1 for illustration). The peak tail current was corrected for the leak current determined during the first short voltage step to -50 mV. After that, the temperature was increased from room to physiological one ($36 \pm 1^\circ\text{C}$) using a programmable Chipholderblock Temperature Controlling Unit.

The cells were then continuously perfused with EC for up to 6 minutes to establish stable pre-drug recordings. After this control phase, drug solutions were continuously applied to the cell via the transport channel for 5 (non-sticky compounds) or for 8 (terfenadine) minutes [12].

Exemplary traces presenting the effects of tested compounds on hERG currents and the voltage protocol are presented in Figure 1.

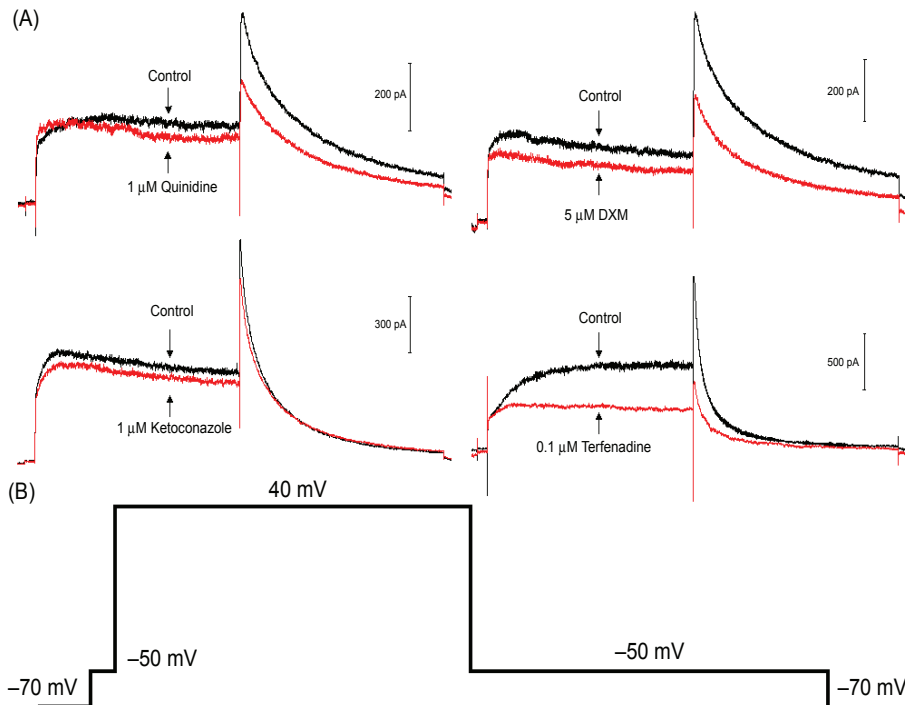


Fig. 1. Sample effects of tested compounds on hERG currents. For each drug, the control current before drug administration is shown in black and the final effect of the drug is shown in red (A). The voltage protocol is depicted below (B).

Data analysis and statistical procedures

Up to three increasing concentrations of the same compound were applied to one cell. Only those cells with a stable whole-cell membrane resistance of more than 500 M Ω and a hERG tail current amplitude of at least 300 pA were used for analysis. hERG tail currents were averaged over 50 s at the end of the control phase and the end of each application phase. Current inhibition was calculated by dividing the mean tail current in the presence of the drug by the mean tail current of the control phase. All measurements were taken at physiological temperature.

For calculation of the concentration-response relationships for the inhibition of hERG peak tail current amplitudes, tested drugs were studied at the following concentrations: 0.1, 0.5, 1, 5, and 10 μM for quinidine; 0.1, 1, 5, and 10 μM for ketoconazole; 0.5, 1, 5, and 10 μM for dextromethorphan; 0.003, 0.03, 0.1, and 0.3 μM for terfenadine. The number of observations ranged from 3 to 7 per test concentration.

Dose-response relationships for each drug were determined by fitting the inhibition of peak tail current amplitudes caused by a concentration C of a given compound, $I(C)$, to the Hill equation (MATLAB R2014a curve-fitting toolbox; MathWorks; Nonlinear Least Squares method and Trust-Region algorithm):

$$I(C) = \frac{100}{1 + (IC_{50}/C)^n}$$

where IC_{50} is the median inhibitory concentration (concentration of a drug that causes 50% current inhibition) and n is the slope parameter (Hill coefficient). Figure 2 shows the obtained results and fitted Hill plots.

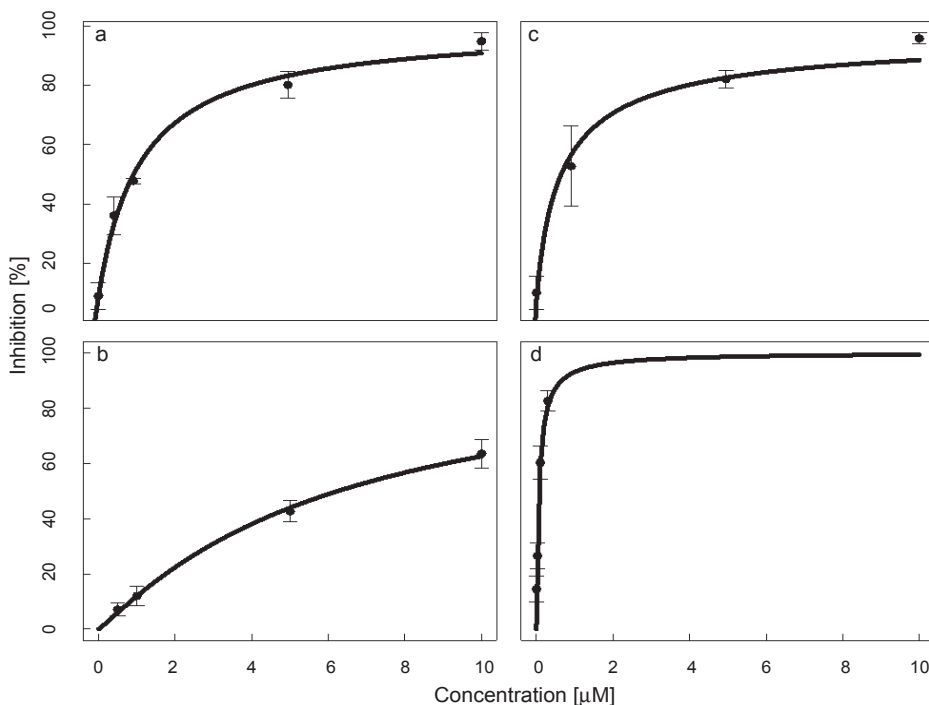


Fig. 2. Fitted dose-response curves (solid lines) and experimentally obtained data (points). Four compounds were tested: quinidine (A), dextromethorphan (B), ketoconazole (C) and terfenadine (D). Error bars show the standard deviation.

In silico study

The Cardiac Safety Simulator (CSS) is a tool for *in vitro-in vivo* extrapolation of the cardiac effects of drugs [2]. The CSS platform allows for the evaluation of the influence of multiple ion channels inhibition on the human left ventricular cardiomyocyte, and then on the cell string of connected cells, which is supposed to mimic the heart wall. The simulated outputs include the action potential (AP), the pseudo-ECG and their derivatives: (1) the APD50 (time needed to reach 50% of repolarization) and the APD90 (time needed to reach 90% of repolarization) for the action potential and (2) the QT, the QRS, the J-Tpeak, and the Tpeak-Tend for the ECG level. The O'Hara-Rudy model, a mathematical description of the electrophysiology of epicardial, endocardial and midmyocardial (M) cells, was utilized during the described study [13].

Table 2. In vitro inhibition data utilized during the simulation study.

Compound	Value	IKr			IKs			INa			ICaL		
		IC ₅₀	n	Ref.	IC ₅₀	n	Ref.	IC ₅₀	n	Ref.	IC ₅₀	n	Ref.
Quinidine	reference_1	0.33	0.74	[15]	44	1*	[27]	14.6	1.22	[14]	6.4	0.68	[14]
	reference_2	0.72	1	[14]									
Ketoconazole	reference_1	1.7	0.9	[28]	-	-	-	-	-	-	-	-	-
DXM	reference_1	5.1	1.02	[29]	-	-	-	-	-	-	-	-	-
Terfenadine	reference_1	0.0066	1*	[30]	4.4	1*	[31]	2	1.81	[15]	0.93	1.8	[15]
	reference_2	0.05	1.15	[15]									

* assumed as 1 due to the lack of information in the respective reference source

Midmyocardial (M) cells are the most sensitive to disruption of ionic current and it was these cells that were used for simulations on the single cell level. Simulation was run until the steady state and the last 12 seconds were considered sufficiently stable to be analyzed. The raw traces were further analyzed for the early afterdepolarization (EAD) presence, as EADs have been proposed as the potential pro-arrhythmic risk surrogates [9]. The second arm of the study was based on the pseudo-ECG simulation. A heterogeneous string of cells was put together with 20% of them coming from the epicardium, 30% from the midmyocardium (M) and the rest (50%) from the endocardium. For a one-dimensional string of cells the forward Euler method was used to integrate model equations and the results were then used to calculate a pseudo-ECG.

The space step and the time step were by default set to $\Delta x = 0.01$ mm and $\Delta t = 0.01$ s, respectively. Physiological parameters describing the virtual individual included plasma ions concentration ($K^+ = 4.06$ mM, $Na^+ = 132.55$ mM, $Ca^{2+} = 2.23$ mM) and parameters describ-

ing cardiomyocytes (volume = $6591 \mu\text{m}^3$, area = $1766 \mu\text{m}^2$, electric capacitance = 46.9 pF and sarcoplasmic reticulum volume = $395.5 \mu\text{m}^3$). Heart wall thickness (i.e. string length) was set to 11 mm . Heart rate value was set to 60 min^{-1} (RR = 1000 ms).

Apart from the in-house measured hERG inhibition values, simulations were run separately for different sets of the *in vitro* inhibition data, as presented in Table 2.

The literature offers multiple IC_{50} values from multiple sources for quinidine and terfenadine. We tested those coming from the same study, done by Kramer *et al.* [14]. We have also considered the lowest known to us IC_{50} value from ref. [15]. For ketoconazole two IC_{50} values were tested. The first one was obtained in experiment carried out in the most physiological conditions (temperature, ions bath concentrations, voltage protocol). The second IC_{50} value was the lowest found. There was only one study available for dextromethorphan.

Literature derived IC_{50} values for the hERG channel obtained at room temperature were further scaled to the physiological conditions with the use of inter-system extrapolation factors [16].

IC_{50} values were normalized by the largest found free effective therapeutic plasma concentration ($\text{ETPC}_{\text{unbound}}$), as presented in Table 3. It was assumed that the plasma concentration is equal to the one at the site of action (ion channel in the heart tissue) and the exposure during simulation studies reached 0 (control), 1, 3, 5, 10, 30, 50, 100, 300, 500, and 1000 times that of $\text{ETPC}_{\text{unbound}}$.

Table 3. Literature derived free effective therapeutic plasma concentration ($\text{ETPC}_{\text{unbound}}$) utilized during the simulation study.

Compound	$\text{ETPC}_{\text{unbound}}$	Description	Ref.
Quinidine	0.9–3.2	–	[14, 32]
Ketoconazole	0.025–0.19	–	[14, 32]
DXM	0.008–0.0217	Extensive metabolizers (EM)	[33]
	0.298–0.851	Poor metabolizers (PM)	
Terfenadine	0.0001–0.00029	Without 3A4 inhibition	[14, 32]
	0.003–0.008	With strong 3A4 inhibition	

The obtained pseudo-ECGs are presented as the logarithm of the ratio $\text{ETPC}_{\text{baseline}} / \text{ETPC}_{\text{threshold}}$, where $\text{ETPC}_{\text{baseline}}$ is the highest free therapeutic ETPC as shown in Table 3, and $\text{ETPC}_{\text{threshold}}$ is the lowest free concentration for which a clear arrhythmic signal appear. The higher the ratio's value, the lower the arrhythmia risk (higher concentration is needed to trigger cardiac event). Conversely, the lower the ratio, the higher the risk of an irregular ECG signal.

All simulations were carried out on a laptop PC with 8Gb RAM and an Intel i5 CPU.

Results

The internally obtained IC_{50} and Hill coefficient (n) values for all tested drugs are presented in Table 4.

Table 4. IC_{50} values and Hill coefficients (n) calculated for the concentration-dependent inhibition of hERG peak tail currents by studied compounds in HEK 293 cells.

Compound	IC_{50} [μ M]	Hill coefficient
Quinidine	1.01	1.00
Ketoconazole	0.47	0.80
DXM	6.27	1.09
Terfenadine	0.07	0.96

They were compared against literature reported values obtained in similar conditions for major parameters (cell model, patch clamp technique), yet with significant differences in the experimental protocol (voltage protocols, ion concentrations, temperature, etc.). Results of such comparison are presented below (Table 5, Fig. 3).

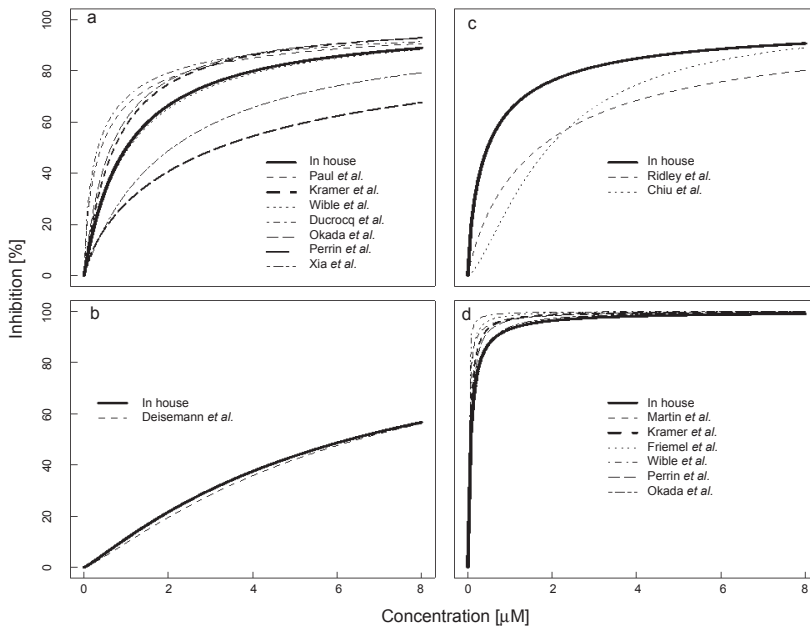


Fig. 3. Dose-response curves for quinidine (a), dextromethorphan (b), ketoconazole (c) and terfenadine (d) for data from Table 4.

Table 5. Representative literature derived and in house measured hERG in vitro inhibition data for four tested compounds.

Compound	Reference	Setup	Cell	IC50 [μ M]	Hill coefficient	Temperature	K ⁺ bath [mM]	t1 pulse [s]	t2 pulse [s]
Quinidine	In house	CytoPatch	HEK	1.01	1.00	P	2.5	2	2
	Paul <i>et al.</i> [34]	Manual	HEK	0.41	0.76	P	4	0.5	0.5
	Kramer <i>et al.</i> [14]	Qpatch or PatchXpress	HEK	0.72	1.06	R	4	2	2
	Wible <i>et al.</i> [35]	Manual	HEK	1.07	1	R	NA	2	2
	Ducrocq <i>et al.</i> [15]	Manual	HEK	0.33	0.74	R	4	1	1
	Okada <i>et al.</i> [36]	Qpatch	CHO	0.638	1.012	R	4	2	2
	Perrin <i>et al.</i> [37]	Manual	CHO	3.2	0.8	R	5	3	0
	Xia <i>et al.</i> [38]	Manual	CHO	2.1	1	R	4	2	1
	In house	CytoPatch	HEK	0.47	0.8	P	2.5	2	2
	Ridley <i>et al.</i> [28]	Manual	HEK	1.7	0.9	P	4	2	4
Ketoconazole	Chiu <i>et al.</i> [39]	Manual	HEK	1.92	1.46	P	4	0.5	0.5
	In house	CytoPatch	HEK	6.27	1.09	P	2.5	2	2
	Deisemann <i>et al.</i> [29]	Manual	CHO	6.5	1.2	R	5.4	2	2
	In house	CytoPatch	HEK	0.07	0.96	P	2.5	2	2
DXM	Martin <i>et al.</i> [40]	Manual	HEK	0.016	0.83	P	5	3	4
	Kramer <i>et al.</i> [14]	Qpatch or PatchXpress	HEK	0.05	1.15	R	4	2	2
Terfenadine	Friemel <i>et al.</i> [41]	Manual	HEK	0.0277	1.13	R	5.6	2	2
	Wible <i>et al.</i> [35]	Manual	HEK	0.008	1	R	NA	2	2
	Perrin <i>et al.</i> [37]	Manual	CHO	0.0641	1.2	R	5	3	0
	Okada <i>et al.</i> [36]	Qpatch	CHO	0.09852	1.176	R	4	2	2

Table 5 shows representative literature and in-house data on tested compounds. Experimental setup (manual or automated patch clamp), cell model (HEK or CHO), conditions (temperature and K^+ ion concentration), and pulse protocols are presented for results with highlighted IC_{50} and Hill coefficient (n) values. P and R stand for physiological and room temperature, respectively.

Figures 4A and B present $\log(ETPC_{baseline}/ETPC_{threshold})$ values for all tested drugs with the proposed safety thresholds separating three risk classes: safe, conditional and

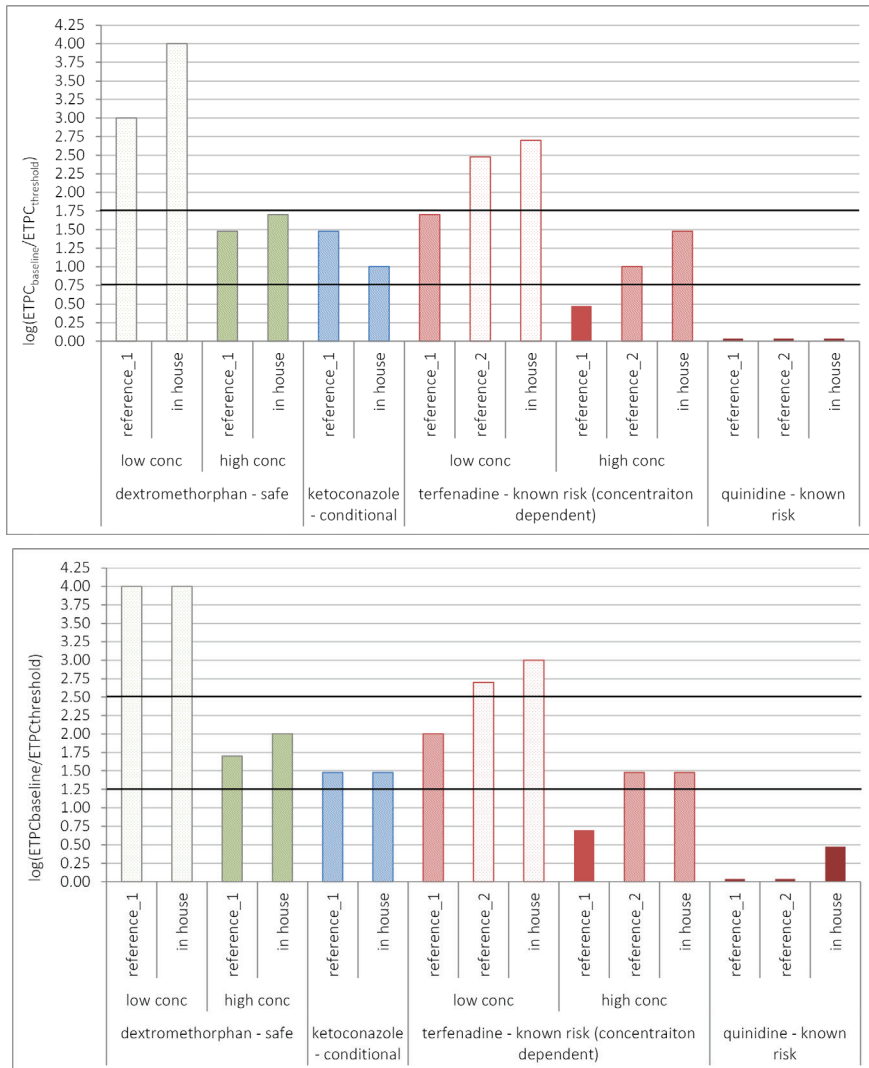


Fig. 4. Log values of the $ETPC_{baseline}/ETPC_{threshold}$ ratios for four tested drugs with the proposed safety thresholds separating three risk classes (A — single cell based simulations and B — pseudoECG).

high risk for the single cell levels and the pseudo-ECG, respectively. It is worth noting that the predicted risk classification depends on the IKr IC_{50} values and on the active concentrations used during the simulation study.

Discussion

As it was previously stated, the aim of the electrophysiological studies done for the purpose of drug cardiac safety assessment is to evaluate the pro-arrhythmic risk. There are multiple factors contributing to the clinically observed, and potentially life-threatening, cardiac arrhythmias [17]. Obviously, not all those factors can be experimentally tested at the pre-clinical stage. This is why *in silico* modeling has become such a popular tool for assessing drug toxicity. By measuring only a small set of *in vitro* parameters (e.g. dose-response relations) and, next, combining them with pharmacokinetic parameters (e.g. ETPC) and system data (human physiology description) it is possible to gain insights into phenomena as complex as the electrical activity of cardiac cells. Even the best theoretical model will fail when fed with biased parameters.

The open question is often which parameters are “biased” and which are “accurate”. Figure 3 clearly shows that even the most basic information on drug-cell interaction, i.e. ion channel inhibition, significantly differs among studies. Dose-response relations obtained from electrophysiological recordings are extremely sensitive to experimental procedures and conditions. Recently, more and more effort has been put into unifying the procedures but still a lot needs to be done [12, 18].

Another question is about using average values that are obtained by either single groups on different *in vitro* setups (e.g. for various temperature conditions or cell types or apparatus) or by different groups that use distinct protocols and equipment. Only large-scale initiatives, bringing together different laboratories for testing the same drug effects in unified conditions, but on different devices, may give insight into the sources of diversity seen in the literature. This is one of the aims of the CIPA initiative [19].

It is imperative that as many conditions as possible are reported in the Methods section for all published reports. Some of them are obvious, like the cell type, temperature, buffer composition and pulse protocols. Others, like the algorithm used to fit the data to the Hill plot or the precise value of not only the IC_{50} , but also the corresponding Hill coefficient, are very often skipped. This makes comparison between datasets impossible. Other parameters that vary between groups are the time of cell incubation with the drug and the time of current recording after drug administration. Especially in automated patch clamp those parameters can greatly affect final results. Simulation studies done with the use of *in vitro* measured values show that the compound specific risk classification may depend on them. In our case this is clearly seen for terfenadine for which proper pro-arrhythmic potency assessment requires thorough analysis. This is because it depends on complex pharmacokinetics (metabolism mainly via cytochrome

P450 3A4) and biological activity (multiple channels inhibition). Terfenadine is a very potent IKr current inhibitor but its biotransformation triggered by the CYP3A enzyme is close to complete in physiological conditions. The first pass metabolism effects in very low systemic concentration of the parent compound but clinically relevant concentration of its main metabolite — fexofenadine which has no effect on the cardiac cells membrane potential and QT interval. Therefore when considered as single drug it should be classified as safe (what is the case for Reference_2 and In house IC50 values but not Reference_1 which over-predicts the risk), whereas for high concentration which can be achieved for example after concomitant exposure to CYP3A4 inhibitors (like ketoconazole) the risk increases what was properly picked-up by simulation done with Reference_1 value as presented in Figure 3. Four substances tested in the current study, although covering a relatively wide range of mechanisms and inhibition activity against various ionic channels, do not allow for the drawing of strong conclusions. We note that in this study we did not attempt to include active metabolites effects or physiological and pathophysiological parameters. Computational power and achievements in the modeling and simulation of cardiac physiology currently allow for *in silico* drug safety testing at the population rather than individual level [20, 21]. Another step would be to account for both sources of uncertainty in the cardiac risk prediction — *in vitro* and physiological factors.

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Conflict of interest

None declared.

References

1. Chi K.R.: Revolution dawning in cardiotoxicity testing. *Nat Rev Drug Discov.* 2013; 12 (8): 565–567.
2. Cardiac safety simulator. Retrieved from <http://www.certara.com/products/cardiac-safety/cardiac-safety-simulator>.
3. Darpo B., Sarapa N., Garnett C., et al.: The IQ-CSRC prospective clinical phase I study: “can early QT assessment using exposure response analysis replace the thorough QT study?”. *Ann Noninvasive Electrocardiol.* 2014; 19 (1): 70–81.
4. Kleiman R.B., Shah R.R., Morganroth J.: Replacing the thorough QT study: Reflections of a baby in the bath water. *Br J Clin Pharmacol.* 2014; 78 (2): 195–201.

5. *Ferber G., Zhou M., Darpo B.*: Detection of QTc effects in small studies--implications for replacing the thorough QT study. *Ann Noninvasive Electrocardiol.* 2015; 20 (4): 368–377.
6. *Darpo B., Benson C., Dota C., et al.*: Results from the IQ-CSRC prospective study support replacement of the thorough QT study by QT assessment in the early clinical phase. *Clin Pharmacol Ther.* 2015; 97 (4): 326–335.
7. *Townsend C.*: Is there a need to add another dimension (time) to the evaluation of the arrhythmogenic potential of new drug candidates in vitro? *Circulation.* 2014; 130 (3): 219–220.
8. *Sager P.T., Gintant G., Turner J.R., Pettit S., Stockbridge N.*: Rechanneling the cardiac proarrhythmia safety paradigm: A meeting report from the cardiac safety research consortium. *Am Heart J.* 2014; 167 (3): 292–300.
9. *Qu Z., Xie L.H., Olcese R., et al.*: Early afterdepolarizations in cardiac myocytes: Beyond reduced repolarization reserve. *Cardiovasc Res.* 2013; 99 (1): 6–15.
10. *CredibleMeds.* AZCERT.org. Retrieved from <https://crediblemeds.org/new-drug-list/>.
11. *Scheel O., Frech S., Amuzescu B., Eisfeld J., Lin K.H., Knott T.*: Action potential characterization of human induced pluripotent stem cell-derived cardiomyocytes using automated patch-clamp technology. *Assay Drug Dev Technol.* 2014; 12 (8): 457–469.
12. *Danker T., Moller C.*: Early identification of hERG liability in drug discovery programs by automated patch clamp. *Front Pharmacol.* 2014; 5: 203.
13. *O'Hara T., Virag L., Varro A., Rudy Y.*: Simulation of the undiseased human cardiac ventricular action potential: Model formulation and experimental validation. *PLoS Comput Biol.* 2011; 7 (5): e1002061.
14. *Kramer J., Obejero-Paz C.A., Myatt G., et al.*: MICE models: Superior to the HERG model in predicting torsade de pointes. *Sci Rep.* 2013; 3: 2100.
15. *Ducroq J., Printemps R., Guilbot S., Gardette J., Salvetat C., Le Grand M.*: Action potential experiments complete hERG assay and QT-interval measurements in cardiac preclinical studies. *J Pharmacol Toxicol Methods.* 2007; 56 (2): 159–170.
16. *Wisniowska B., Polak S.*: hERG in vitro interchange factors-development and verification. *Toxicol Mech Methods.* 2009; 19 (4): 278–284.
17. *Polak S., Pugsley M.K., Stockbridge N., Garnett C., Wisniowska B.*: Early drug discovery prediction of proarrhythmia potential and its covariates. *AAPS J.* 2015; 17 (4): 1025–1032.
18. *Fermini B., Hancox J.C., Abi-Gerges N., et al.*: A new perspective in the field of cardiac safety testing through the comprehensive in vitro proarrhythmia assay paradigm. *J Biomol Screen.* 2015; Epub 2015 Jul 13.
19. CIPA — comprehensive in vitro proarrhythmia assay. Retrieved from <https://www.ilsixtra.org/hesi/science/cardiac/cipa/SitePages/Home.aspx>.
20. *Sarkar A.X., Christini D.J., Sobie E.A.*: Exploiting mathematical models to illuminate electrophysiological variability between individuals. *J Physiol.* 2012; 590 (Pt 11): 2555–2567.
21. *Glinka A., Polak S.*: The effects of six antipsychotic agents on QTc--an attempt to mimic clinical trial through simulation including variability in the population. *Comput Biol Med.* 2014; 4720–4726.
22. *Schadel M., Wu D., Otton S.V., Kalow W., Sellers E.M.*: Pharmacokinetics of dextromethorphan and metabolites in humans: Influence of the CYP2D6 phenotype and quinidine inhibition. *J Clin Psychopharmacol.* 1995; 15 (4): 263–269.
23. *Kaplan B., Buchanan J., Krantz M.J.*: QTc prolongation due to dextromethorphan. *Int J Cardiol.* 2011; 148 (3): 363–364.
24. *Hondeghem L.M., Dujardin K., Hoffmann P., Dumotier B., De Clerck F.*: Drug-induced QTC prolongation dangerously underestimates proarrhythmic potential: Lessons from terfenadine. *J Cardiovasc Pharmacol.* 2011; 57 (5): 589–597.
25. *Crumb W.J. Jr, Wible B., Arnold D.J., Payne J.P., Brown A.M.*: Blockade of multiple human cardiac potassium currents by the antihistamine terfenadine: Possible mechanism for terfenadine-associated cardiotoxicity. *Mol Pharmacol.* 1995; 47 (1): 181–190.

26. *el-Sherif N., Bekheit S.S., Henkin R.*: Quinidine-induced long QTU interval and torsade de pointes: Role of bradycardia-dependent early afterdepolarizations. *J Am Coll Cardiol.* 1989; 14 (1): 252–257.
27. *Kang J., Chen X.L., Wang L., Rampe D.*: Interactions of the antimalarial drug mefloquine with the human cardiac potassium channels KvLQT1/minK and HERG. *J Pharmacol Exp Ther.* 2001; 299 (1): 290–296.
28. *Ridley J.M., Milnes J.T., Duncan R.S., et al.*: Inhibition of the HERG K⁺ channel by the antifungal drug ketoconazole depends on channel gating and involves the S6 residue F656. *FEBS Lett.* 2006; 580 (8): 1999–2005.
29. *Deisemann H., Ahrens N., Schlobohm I., Kirchhoff C., Netzer R., Moller C.*: Effects of common antitussive drugs on the hERG potassium channel current. *J Cardiovasc Pharmacol.* 2008; 52 (6): 494–499.
30. *Kirsch G.E., Trepakova E.S., Brimecombe J.C., et al.*: Variability in the measurement of hERG potassium channel inhibition: Effects of temperature and stimulus pattern. *J Pharmacol Toxicol Methods.* 2004; 50 (2): 93–101.
31. *Lacerda A.E., Kramer J., Shen K., Thomas D., Brown A.M.*: Comparison of block among cloned cardiac potassium channels by non-antiarrhythmic drugs. *European Heart Journal Supplements.* 2001; 3 (Supplement 3): K23–30.
32. *Braam S.R., Tertoolen L., van de Stolpe A., Meyer T., Passier R., Mummery C.L.*: Prediction of drug-induced cardiotoxicity using human embryonic stem cell-derived cardiomyocytes. *Stem Cell Res.* 2010; 4 (2): 107–116.
33. Dextrometorphan. Retrieved from <http://www.nhtsa.gov/people/injury/research/job185drugs/dextromethorphan.htm>.
34. *Paul A.A., Witchel H.J., Hancox J.C.*: Inhibition of the current of heterologously expressed HERG potassium channels by flecainide and comparison with quinidine, propafenone and lignocaine. *Br J Pharmacol.* 2002; 136 (5): 717–729.
35. *Wible B.A., Hawryluk P., Ficker E., Kuryshv Y.A., Kirsch G., Brown A.M.*: HERG-lite: A novel comprehensive high-throughput screen for drug-induced hERG risk. *J Pharmacol Toxicol Methods.* 2005; 52 (1): 136–145.
36. *Okada J.I., Yoshinaga T., Kurokawa J., et al.*: Screening system for drug-induced arrhythmogenic risk combining a patch clamp and heart simulator. *Sci Adv.* 2015; 1 (4): e1400142.
37. *Perrin M.J., Kuchel P.W., Campbell T.J., Vandenberg J.I.*: Drug binding to the inactivated state is necessary but not sufficient for high-affinity binding to human ether-a-go-go-related gene channels. *Mol Pharmacol.* 2008; 74 (5): 1443–1452.
38. *Xia M., Shahane S.A., Huang R. et al.*: Identification of quaternary ammonium compounds as potent inhibitors of hERG potassium channels. *Toxicol Appl Pharmacol.* 2011; 252 (3): 250–258.
39. *Chiu P.J., Marcoe K.F., Bounds S.E., et al.*: Validation of a [³H]astemizole binding assay in HEK293 cells expressing HERG K⁺ channels. *J Pharmacol Sci.* 2004; 95 (3): 311–319.
40. *Martin R.L., McDermott J.S., Salmen H.J., Palmatier J., Cox B.F., Gintant G.A.*: The utility of hERG and repolarization assays in evaluating delayed cardiac repolarization: Influence of multi-channel block. *J Cardiovasc Pharmacol.* 2004; 43 (3): 369–379.
41. *Friemel A., Zunkler B.J.*: Interactions at human ether-a-go-go-related gene channels. *Toxicol Sci.* 2010; 114 (2): 346–355.