Quantification of hERG Potassium Channel Block from the ECG

Johan de Bie[1], W. Brian Chiu[1], David W. Mortara[2] , Cristiana Corsi[3], Stefano Severi[3]

[1]Mortara Instrument, Inc., Milwaukee, United States of America
[2]University of California, San Francisco, United States of America
[3]Università di Bologna, Cesena, Italy

Abstract

Blood potassium concentration ($[K^+]_{blood}$) influences the ECG T-wave morphology. T-wave slope-to-amplitude ratio ($T_{S/A}$) has been shown to be correlated with $[K^+]_{blood}$, suggesting that it is a metric for the repolarizing I_{Kr} current, which flows through the hERG ion channels in cardiac cells. In this study we tested whether $T_{S/A}$ can also be used as an alternate ECG-based metric of the amount of hERG block in drug safety trials.

We used data from two FDA-sponsored placebo-controlled cross-over studies, in which healthy subjects received a single or multiple doses of hERG-blocking drugs, either alone or in combination with compounds that block late sodium or calcium ion channels. $T_{S/A}$ was compared with other ECG markers for the ability to measure the amount of hERG block independent from other confounding ion channel block of the same drug or a combination of drugs. $T_{S/A}$ appeared to be exclusively sensitive to hERG block and independent of the drug type. It is a more accurate and stable ECG metric for hERG block than T_{peak}-T_{end}, $LRD_{30\%}$ and QTc (which is also sensitive to late sodium block). We conclude that $T_{S/A}$ should be added to the set of ECG markers measured in phase I drug safety trials.

1. Introduction

Drug-induced QT prolongation increases the risk for torsade de pointes, a potentially fatal ventricular arrhythmia, and has resulted in drugs being removed from the market [1]. The current regulatory paradigm for assessing drug cardiac safety, which is extremely focused on QT prolongation, is preventing potentially effective medicines from reaching the market, sometimes inappropriately [1]. To address this safety issue, the US Food and Drug Administration (FDA) and multiple public-private partnerships are studying novel approaches with a Comprehensive in vitro Proarrhythmia Assay and in Phase 1 clinical trials [2,3]. Essential to the novel approaches is a focus on the effects of drugs on multiple cardiac ion channels, which can be either pro- or anti-arrhythmic, depending on the combination.

Almost all drugs that may cause torsade de pointes block the hERG potassium channel. As a consequence, the outward potassium current is reduced at the cellular level and the QT interval is prolonged on the electrocardiogram (ECG) [4]. However, for some of these drugs the torsade de pointes risk is minimal [5].

In addition to the QTc interval (heart rate corrected QT), other ECG biomarkers have been introduced to measure the effect of ion channel blocks. Of note, in the context of this paper, is the ECG-interval from the T-wave peak to its end (T_pT_e). hERG block also affects the morphology of the T-wave; various markers have been proposed to measure those changes, which cause some variability in the measurement of T-wave peak, needed for T_pT_e, and in general making its significance difficult to interpret.

Hypokalemia in some respects mimics hERG channel block in that it also causes a reduced potassium outflow current. Besides QT prolongation, classic ECG hallmark of hypokalemia is a reduced T-wave amplitude [6], while the opposite (hyperkalemia) is traditionally diagnosed as tall, peaked T-waves [7]. We recently proposed a metric to quantify the plasma potassium concentration [8], based on the ratio of down-going T-wave slope and T-wave amplitude ($T_{S/A}$) and tested it on patients undergoing haemodialysis. The relationship between this metric and blood plasma potassium concentration suggested that the metric could actually measure outward potassium current. This hypothesis was confirmed by model studies and measurements on LQT2-patients [8]. Incidentally, the same study showed that $T_{S/A}$ was not sensitive to the, sometimes extensive, calcium (Ca) and sodium (Na) concentration changes during the dialysis. The observation has led us to evaluate $T_{S/A}$ in the context of drug safety studies as an alternate metric of the amount of hERG block, independent from Na and Ca channel block.

 ISSN: 2325-887X DOI:10.22489/CinC.2017.239-123

2. Method

We used data from two previous clinical studies, both similar in design to dedicated QT studies [9], in which 22 young volunteers without ECG or apparent cardiovascular abnormalities participated. The studies were designed as a randomized, double-blind, 5-period crossover clinical trials at a phase 1 clinical research unit (Spaulding Clinical, West Bend, WI). Subjects were "dosed" with a combination of drugs or placebo, depending on the trial, either at the beginning of the 24 hour measurement period or at various time points in the morning, afternoon and evening, and blood samples for drug concentration measurements were taken at various time points. Subjects repeated the recording period at consecutive weeks to complete the 5 arms of the study. During each period, continuous ECGs were recorded at 500 Hz and with an amplitude resolution of 2.5 μV (Mortara Instrument, Milwaukee). From the continuous recording, triplicate 10-second ECGs were extracted at predose and 15 other time points including those of the blood draws using Antares software (AMPS-LLC, New York City). The studies were approved by the U.S. FDA Research Involving Human Subjects Committee and the local institutional review board. All subjects gave written informed consent. ECG's and drug concentrations have been made available in Physionet [10]:
https://physionet.org/physiobank/database/ecgrdvq/.

The first study has been described in [11,12]. The study is identified in the data description file in Physionet and this paper as "002". The second study, identified as "003", uses different drugs and doses [13]; see:
https://physionet.org/physiobank/database/ecgdmmld/.

We re-analyzed all ECG's from the raw data samples with the Mortara Instrument VERITAS resting ECG interpretation program, to which we had added a module to calculate the $T_{S/A}$ parameter. The principle of the calculation has been described previously [8,14]. For this study, we used the vector magnitude of all independent leads as input for the calculation, but similar results were obtained using only leads I and II.

We compared $T_{S/A}$ with a diverse number of ECG-metrics which were provided for each ECG in the data description files available on Physionet. Their meaning is described in [11-13] We focused on the metrics that resulted from these previous studies as mostly sensitive to hERG block, and the least affected by sodium (Na) blocking agents [16]: $LRD_{30\%}$ (the time it takes for the T-vector amplitude to decrease 30% after the peak) and the T_pT_e; as a reference we used the QTc interval. For RR-correction of the QT-interval, Fredericia's correction ($QTc = QT/(RR/1000\ ms)^{1/3}$) was used. Other parameters, including $T_{S/A}$ were not corrected for RR. For all measurements, we have taken the median of the three ECG's provided at each time point.

From each drug plasma concentration, we calculated the estimated %hERG block with the Hill equation:

$$B(\%) = 100 \cdot \frac{D^n}{IC_{50}^n + D^n}$$

where B(%) is the percentage of current blockage at drug concentration D, IC_{50} is the concentration of drug that causes 50% block, and n is the Hill coefficient. The IC_{50} and n values for each drug were obtained from data in [16]. In the case of moxifloxacin, the effect of its glucuronide metabolite (M2) that exhibits hERG potassium channel block has been taken into account: based on previous observations [13,17] we considered for M2 a 5% protein binding and hERG block parameters equal to those of the parent drug.

We used 0% block for all time points with placebo and for those where only drugs with no known hERG block were used, and we did not use any data points without available plasma concentrations.

In order to exclude any subject and time-of-day dependencies, we applied a "Delta-Delta" (ΔΔ) design, that is, we subtracted from each time point the values for the same subject at pre-dose, as well as the values for the "Delta" at corresponding time points in the placebo arm.

3. Results

Study 002 was designed as a single dose study that sampled the subject during the following gradual increase and subsequent decrease of drug plasma concentration. Four drugs were used: dofetilide, a pure hERG channel blocker; quinidine, with stronger hERG channel block and, at higher concentrations, also some calcium (Ca) and Na channel blocks; ranolazine with a moderate hERG block together with an equal amount of Na channel block; verapamil with little hERG block but a higher amount of Ca channel block. An example of a scatter plot of the ΔΔ samples as a function of %hERG block (calculated from the measured plasma concentration) for QT_c and $T_{S/A}$ in the quinidine arm of the study is shown in Fig. 1.

Some of the metrics were clearly non-linear to %hERG block, and also the spread around the mean depends on the drug and the actual %hERG block. In order to compare metrics under various %hERG block, we divided the data points (e.g. those in Fig. 1) into four groups: little or no (<10%) block, small (10-30%) block, medium (30-50%) block, and large (>50%) block. We then calculated the average value of each of the metrics in each group. As a measure of confidence, we calculated the standard deviation (SD) of the difference between the data and a linear regression line through the origin, per group. We converted the response of all metrics to "%hERG" using the response to the medium (30-50%) amount of hERG block by dofetilide, a pure potassium blocker, as a normalizing scale factor.

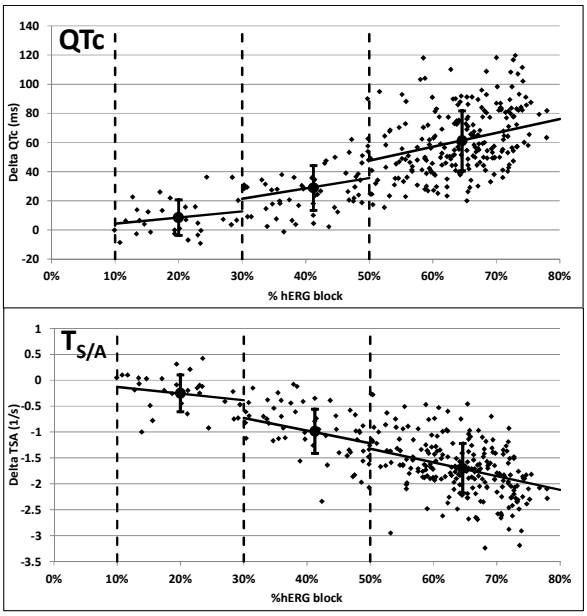

Figure 1: Scatter plot of the ΔΔ-QTc interval and $T_{S/A}$ as a function of %hERG block by quinidine. Vertical dotted lines indicate grouping; solid lines are linear regression forced through the origin for each group; centered large dots are the average for each group and the vertical standard deviation of the error with respect to the regression line.

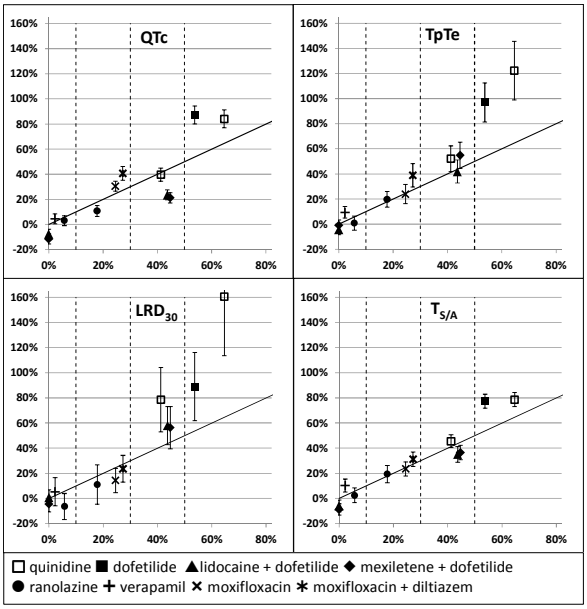

Figure 2: ECG-indicators converted to estimated %hERG block as a function of measured block. Solid line indicates unity response, vertical bars are the 95% confidence interval of a 60 data point sample.

Study 003 had a more complicated dosing scheme [17] which resulted in time points being available only around the period of maximum plasma concentration of any given drug combination. Arms 1-3 used dofetilide as the hERG channel blocker, by itself, or in combination with lidocaine or mexiletine, mostly Na channel blockers. The morning dose did not contain any dofetilide; the afternoon and evening doses contained increasing amounts of all drugs. In the fourth arm, moxifloxacin was used, to which diltiazem was added in the evening dose. Moxifloxacin mostly blocks the hERG potassium channels, while diltiazem is a Ca channel blocker. We did the same analysis as for the previous study data. We found that the %hERG gain for dofetilde was somewhat lower than in the previous study for all markers, so we recalibrated the translation to %hERG block by the response to dofetilide.

Combined results can be seen in Figure 2. All groups from both studies with at least 60 raw data samples are shown, except the response to 30-50%hERG block by dofetilide alone, used for normalization. The standard deviations are scaled such that they indicate the 95% confidence interval of a 60 data point sample.

In Table 1, the SD of each of the metrics is shown as a function of the amount of %hERG block.

Table 1: SD of the %hERG block estimate by several ECG markers for various amounts of block

%hERG	QTc	TpTe	LRD30%	TSA
<10%	14%	18%	31%	20%
10-30%	17%	24%	47%	21%
30-50%	20%	38%	61%	24%
>50%	26%	56%	116%	24%
Average	19%	34%	64%	22%

4. Discussion and Conclusions

The response of all ECG metrics to pure hERG blockers (dofetilide and quinidine) is non-linear: the ECG-estimate at high plasma concentrations is clearly too high (Fig. 2 ■ and □, group>50%). $T_{S/A}$ has less over-estimation than the other metrics. $LRD_{30\%}$ and T_pT_e particularly overestimate at high quinidine concentrations.

As is known from previous work (13,15), QTc consistently under-estimates hERG block in the presence of sodium blockers (Fig 2, ● ranolazine, ▲ lidocaine or ◆ mexiletine,), while $T_{S/A}$, T_pT_e and $LRD_{30\%}$ do not..

When normalized by dofetilide, $LRD_{30\%}$ underestimates the amount of hERG block of moxifloxacine (Fig 2, ✕). The addition of the calcium blocker diltiazem increased QTc, T_pT_e and $LRD_{30\%}$, but $T_{S/A}$ was affected little (Fig 2, ✱).

A low variability is important for the ECG biomarkers, in order to keep the number of subjects in a study as low as possible. It can be seen from table 1 that QTc and $T_{S/A}$ have similar average standard deviations, but T_pT_e is 50% higher, and $LRD_{30\%}$ three times as high. This is caused in particular by the cases of a higher amount of hERG block. The variability of $T_{S/A}$ is not affected by %hERG block.

In conclusion, $T_{S/A}$ clearly outperforms the other two ECG metrics T_pT_e and $LRD_{30\%}$ as a measure of %hERG block. $T_{S/A}$ is not affected by Na and Ca blockers, and independent of the type of drug, $T_{S/A}$ is more stable than T_pT_e and $LRD_{30\%}$, in particular at high amounts of %hERG block where variation in T-wave morphology is present, like with quinidine.

$T_{S/A}$ is a fully automatically calculated metric. It is stable and not affected by morphology changes like notches and double peaks because there is no need to precisely measure the timing of landmarks like T-wave peak or end. The measurement is robust and not susceptible to artifact. Like T_pT_e and $LRD_{30\%}$, the metric focuses on the latter part of the T-wave and, like T_pT_e and $LRD_{30\%}$, its dimension is that of (inverted) time.

We propose that $T_{S/A}$ be one of the metrics used in early Phase 1 exposure-response modeling studies to determine if there are unexpected effects compared to preclinical ion channel data, which might occur due to a human-specific metabolite or protein binding, as part of the Comprehensive in vitro Proarrhythmia Assay (CiPA) paradigm [2].

Acknowledgements

The authors wish to thank Jose Vicente from the USA Food and Drug Administration for his valuable advice.

References

[1] Stockbridge N, Morganroth J, Shah RR, et al. Dealing with global safety issues: was the response to QT-liability of non-cardiac drugs well-coordinated? Drug Saf, 2013;36:167-182.

[2] Sager PT, Gintant G, Turner JR, et al. The cardiac proarrhythmia safety paradigm: a meeting report from the Cardiac Safety Research Consortium. Am Heart J, 2014;167:292–300.

[3] Darpo B, 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;97:326–335.

[4] Redfern WS, et al. Relationships between preclinical cardiac electrophysiology, clinical QT interval prolongation and torsade de pointes for a broad range of drugs: evidence for a provisional safety margin in drug development. Cardiovasc Res, 2003;58:32–45.

[5] Martin RL, McDermott JS, Salmen HJ, et al. The utility of hERG and repolarization assays in evaluating delayed cardiac repolarization: influence of multi-channel block. J Cardiovasc Pharmacol, 2004;43:369–379.

[6] Greenlee M, Wingo CS, McDonough AA, et al. Narrative review: evolving concepts in potassium homeostasis and hypokalemia. Annals of Internal Medicine, 2009;150:619-625.

[7] Lehnhardt A, Kemper MJ Pathogenesis, diagnosis and management of hyperkalemia. Pediatric Nephrology, 2011;26:377–384.

[8] Corsi C, Cortesi M, Callisesi G, et al. .Noninvasive quantification of blood potassium concentration from ECG in hemodialysis patients. Scientific Rep, 2017;7:#42492.

[9] US Food and Drug Administration. Guidance for Industry E14 Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for Non-Antiarrhythmic Drugs. 2005.

[10] Goldberger AL, Amaral LAN, Glass L, et al. PhysioBank, PhysioToolkit, and PhysioNet: Components of a New Research Resource for Complex Physiologic Signals. Circulation, 2000;101(23):e215-e220.

[11] Johannesen L, Vicente J, Mason JW, et al. Differentiating Drug-Induced Multichannel Block on the Electrocardiogram: Randomized Study of Dofetilide, Quinidine, Ranolazine, and Verapamil. Clin Pharmacol Ther, 2014;96(5):549-58.

[12] Vicente J, Johannesen L, Mason JW, et al. Comprehensive T wave morphology assessment in a randomized clinical study of dofetilide, quinidine, ranolazine, and verapamil. J Am Heart Assoc, 2015;4:e001615.

[13] Johannesen L, Vicente J, Mason JW, et al. Late sodium current block for drug-induced long QT syndrome: Results from a prospective clinical trial. Clin Pharmacol Ther, 2016;99(2):214-23.

[14] Corsi C, De Bie J, Napolitano C, et al. Validation of a Novel Method for Non-invasive Blood Potassium Quantification from the ECG. Computing in Cardiology 2012;39:105-108.

[15] Vicente J, Johannesen L, Hosseini M, et al. Electrocardiographic Biomarkers for Detection of Drug-Induced Late Sodium Current Block. PLoS One, 2016;11(12): e0163619.

[16] Crumb WJ, Jr., Vicente J, Johannesen L, et al. An evaluation of 30 clinical drugs against the comprehensive in vitro proarrhythmia assay (CiPA) proposed ion channel panel. J Pharmacol Toxicol Methods, 2016. 81: 251-62.

[17] Stass H, Kubitza D. Pharmacokinetics and elimination of moxifloxacin after oral and intravenous administration in man. J Antimicrob Chemother, 1999:43(suppl. B):83–90.

Address for correspondence.

Johan de Bie,
Mortara Instrument Europe srl
Via Cimarosa 103,40033 Casalecchio di Reno (BO), Italy
Johan.debie@mortara.com