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Improving the Detection of Subtle I_{Kr}-Inhibition

Assessing Electrocardiographic Abnormalities of Repolarization Induced by Moxifloxacin

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Abstract

Background: QT prolongation is an incomplete measure of drug-induced changes in repolarization. In this study, we investigated a novel, automatic ECG technique for describing ventricular repolarization morphology and we compared these results to corrected QT (QTc) prolongation for identifying ECGs of healthy individuals on moxifloxacin.

Methods: We analysed data from the US FDA ECG Warehouse involving 160 standard ECGs from 40 healthy subjects enrolled in a randomized, parallel, placebo-controlled, 'thorough QT' study. Computerized ECG analysis included a series of scalar and vectorial parameters describing duration of repolarization segments and T-wave/loop morphology including its symmetry, amplitude and shape. Binary logistic models for the identification of moxifloxacin-induced abnormalities of the repolarization were developed.

Results: Moxifloxacin induced significant changes in several ECG parameters including QT and QT apex and early repolarization duration $(ERD)_{30\%}$, T-wave amplitude and slopes of the ascending and descending arm of the T-wave. The logistic model based only on T-wave morphology parameters outperformed the model based on QTc interval for identifying the presence of moxifloxacin. Combining information about repolarization interval duration with T-wave morphology significantly improved the detection of presence of moxifloxacin ($p < 0.01$). The increased sensitivity of our novel ECG method contributes to a >40% reduction in the sample size required to detect significant QTc prolongation induced by moxifloxacin.

Conclusions: Repolarization morphology is significantly altered by moxifloxacin. The computerized ECG technique provides a novel method for quantifying morphological changes of repolarization segment. Our new parameters reflecting the morphology of the T-wave outperformed QTc measurements when identifying moxifloxacin-induced blockade of the outward rapid components of the delayed

rectifier repolarizing potassium current (I_{Kr}). These data indicate that the analysis of T-wave morphology could play a role in the assessment of drug toxicity.

Background

Prolongation of the QT interval from the surface ECG is currently used as a surrogate marker of the potential torsadogenic properties of cardiac and non-cardiac drugs.[1] Following several cases of cardiac death reported in individuals on the non-cardiac drugs terfenadine[2] and cisapride,[3] the US FDA currently requires from pharmaceutical companies that all new compounds should be tested for their potential QT prolonging effects. This safety assessment relies on results from a clinical trial specifically designed to identify small drug-induced QT prolongation effects from surface ECGs in healthy individuals: the so-called 'thorough QT' studies. Uniquely relying on the measurements of QT prolongation, these clinical trials generally follow a placebo-controlled, cross-over design. They include a positive control group used to validate the QT interval measurement method (no specific QT measurement technique is recommended).[4] The positive control group consists of healthy individuals receiving moxifloxacin.[5,6]

Moxifloxacin is a drug from the class of fluoroquinolone antibacterials and is associated with a small repolarization delay (around 5 ms).[7] In the general population, a normal dose (400 mg) of moxifloxacin is considered safe with a very low rate of torsades de pointes (TdP).[5,8] The QT prolongation effect of moxifloxacin is linked to a reduction of the outward rapid component of the delayed rectifier potassium current (I_{Kr}) of the myocardial cells.[9]

Almost all drugs that have been associated with TdP modify the kinetics of this specific ion current.[10] However, while several drugs that have no history of cardiac events will prolong the QT interval, others will be associated with very small prolongation and torsadogenic properties. Thus, it is recognized that the risk of TdP is not a function of the QT interval, nor of the extent of the QT-interval prolongation during drug therapy.[11] Consequently, the FDA faces a challenging issue related to the validity of using QT interval measurements for evaluating the safety level of new drugs, and the Agency recognizes the need for more precise and more meaningful electrocardiographic markers.

In the hereditary and the induced-form of the long QT syndrome (LQTS), several studies have reported the presence of various repolarization changes related to T-wave morphology on the surface ECGs.[12-14] The value of such morphological abnormalities for the prediction of an increased risk of arrhythmic events remains to be elucidated, but any ECG technique revealing the presence of a drug with I_{Kr}-blocking properties could potentially improve the current safety assessment methods, which at present depend solely on heart rate corrected QT (QTc) prolongation.

In this study, we designed and investigated a set of new ECG parameters to specifically quantify delay within the ventricular repolarization process measured on the surface ECGs, but independent from the location of the end of the T-wave. We used a technique based on the principal component analysis of the repolarization signals, and measured specific intervals in the vectorial repolarization loop. We compared the ability of these new parameters to identify the presence of moxifloxacin in healthy individuals and their precision in comparison to the semi-automatic QT interval measurements.

Method

Study Population

This retrospective study relied on ECG tracings from 40 individuals recorded during a thorough QT study. The recordings were extracted from the FDA ECG Data Warehouse. This study was a parallel, placebo-controlled study including 18 females. The exclusion criteria when enrolling the individual in the study were the presence of pregnancy, diabetes mellitus, any cardiac history or a QTc interval dura-

tion >470 ms. Inclusion criteria required normal value ranges for body mass index, laboratory screening and ECG tracings.

From the FDA, we obtained four digital ECG recordings per individual acquired over 2 days. Each day, the two ECGs were recorded at 0700 h and 0900 h. Day 1 was a baseline day. During day 2, subjects received a single dose of moxifloxacin 400 mg (n = 20) or placebo (n = 20). The moxifloxacin dose and the placebo were given just after the first ECG on day 2. The second ECG on day 2 was recorded 2 hours after drug or placebo administration at the expected time of maximum concentration of moxifloxacin (0900 h). One hundred and sixty ECGs from the baseline, placebo and moxifloxacin arms were analysed.

ECG Technology

The ECG equipment used in this thorough QT study was the ELI 100 recorder (Mortara Instrument, Milwaukee, WI, USA). The ECG digital files were available in the HL7 XML ECG format. The technical specifications of the signal were 500 Hz sampling frequency and an amplitude resolution of 6.25 μV/bit.

Scalar ECG Measurements

The measurements of the RR intervals and repolarization intervals were based on technology developed at the University of Rochester Medical Center, Rochester, NY, USA. The COMPAS software provided the identification of the end of the T-wave on lead II based on a technique identifying the crossing point between the baseline and the descending slope of the T-wave (least-squares technique).[15] The end of the T-wave was visually checked by trained technicians and manually adjusted using the COMPAS on-screen caliper if the automatic algorithm failed to correctly identify the end of the T-wave (semi-automatic method).

All measurements and the manual adjustment of the QT interval durations were done in a blinded fashion. The QT interval measurements were done in all available sinus beats of lead II and the median value was reported. The apex of the T-wave relied on a method using a parabola fit of the T-wave where the maximum of the parabola identified the location of the apex. The TpTe intervals was computed from QT and QT apex intervals such as TpTe = QT − QT apex. Baseline wandering was adjusted using Spline interpolation. The amplitude of the T-wave was measured at the apex of the T-wave.

The right (αR) and left (αL) slopes of the T-wave, expressed in μV/ms, were computed using the least-square regression method fitting the up and down slopes of the T-wave.[16] The symmetry of the T-wave was measured by the ratio of the left to right slope of the T-wave (Tsym = |αL/αR|; no unit).

Vectocardiographic Measurements

The vectorcardiographic measurements were based on the principal component analysis of the repolarization segment defined between the J point and the point located 220 ms before the next R peak. This ensures that (i) the analysis encompasses all components of the ventricular repolarization signal; and (ii) it is independent from the determination of the end of the T-wave. Such a method requires the individuals or patients to be at rest.

The vectocardiographic parameters measure the duration of a set of intervals inside the T-Ioop when it is represented in its preferential plane. This preferential plane is defined by the two first eigenvectors computed from the principal component analysis. The method has been described previously.[17] The starting point of these intervals is the time at which the length of the repolarization heart vector is maximized (Vmax; shown in the upper panel of figure 1). The ending points of these intervals are delimited by a circle of diameter equal to 30% (for early repolarization duration $[ERD]_{30\%}$ and late repolarization duration $[LRD]_{30\%}$) of Vmax as illustrated in figure 1. Consequently, these parameters measure the time needed for the repolarization vector to vary from its maximum length to a time point corresponding to a 30% reduction of its maximum length. $ERD_{\%}$ measures an interval directed toward the QRS complex whereas $LRD_{\%}$ measures an interval toward the end of the repolarization interval. The duration of these intervals increase when the electrical vector slows

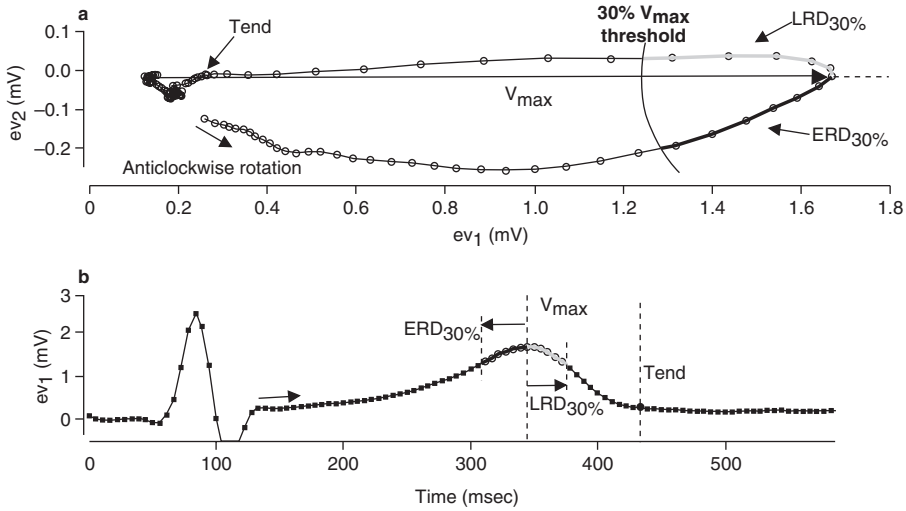

Fig. 1. Measurements of the early repolarization duration $(ERD)_{30\%}$ and late repolarization duration $(LRD)_{30\%}$ intervals. (**a**) This panel describes the T-loop in its preferential plane defined by the two first eigenvectors. The longest arrow marks the maximum vector (Vmax) of the loop; $ERD_{30\%}$ and $LRD_{30\%}$ measure the time needed for this Vmax to be reduced by 30%. ERD is directed toward the preceeding QRS complex whereas LRD is directed toward the end of the T-wave. (**b**) Description of the location of these intervals defined by $ERD_{30\%}$ and $LRD_{30\%}$ on the cardiac signal (first eigenvector). **ev** = eigenvector.

down and/or the roundness of the T-loop increases. The total repolarization duration (TRD) is the sum of ERD and LRD. Finally, we report the repolarization complexity and the planarity of the loop corresponding to the ratio of the two first eigenvalues and the normalized third eigenvalue.

Heart Rate Correction

All repolarization measurements were heart-rate corrected using the pooled technique. A linear regression analysis was used to model the relationship between repolarization measurements and RR intervals during baseline periods (80 tracings). The slope (β) characterizing this relationship was used to correct the repolarization measurements for the QT interval as follows (equation 1):

$$QTc = QT + \beta.(1 - RR) \quad \text{(Eq. 1)}$$

The same heart-rate correction technique was applied to all other measurements. The QT interval durations were also corrected for heart rate using Fridericia's (QTcF) and Bazett's (QTcB) formulae.

Statistical Analysis

For each ECG, we measured the repolarization parameters in all cardiac beats and then computed their average values amongst all beats. For each individual, we computed the differences of values of repolarization parameters from ECGs on treatment versus baseline ($\Delta_{moxi\text{-}baseline}$ and $\Delta_{placebo\text{-}baseline}$). The moxifloxacin effect was controlled for the placebo effect by subtracting the average placebo effect from the average moxifloxacin effect, thereby yielding a single mean value per ECG parameter (equation 2):

$$\Delta\Delta = \text{mean}(\Delta_{moxi\text{-}baseline}) - \text{mean}(\Delta_{placebo\text{-}baseline}) \quad \text{(Eq. 2)}$$

The mean($\Delta_{moxi\text{-}baseline}$) and mean($\Delta_{placebo\text{-}baseline}$) and their standard error were computed for each repolarization parameter. The non-parametric Wilcoxon rank-sum test was used to compare groups. We used binary logistic regression models to describe the association between the presence of moxifloxacin and the changes in ECG parameters. We performed both univariate and multivariate analyses. Best subsets regression was used to select optimal models. Model comparisons were based on

the Akaike Information Criterion (AIC). This criterion penalizes the log-likelihood of the model for each additional parameter.[18] Receiver operating characteristic (ROC) curves (displaying 'sensitivity' vs '100-specificity' in percentage) were constructed to investigate the predictive power of fitted models. In particular, we used the area under the ROC curve (AUC) as a criterion for model comparison.

Comparison of selected models was done using the likelihood ratio (LR) test when models were nested; that is, the set of independent variables making up one model forms a subset of the set of independent variables making up the second model, or vice versa. To the best of our knowledge, no valid formal statistical test has been proposed that we could use to compare no-nested models. To mitigate this problem, we fitted a logistic regression model including as covariates both the predictors that define the model of reference and those defining the model to which it was compared.

The variability and reproducibility of the repolarization parameters were investigated using only the baseline recordings; thus, restricting our study to untreated individuals. Each individual had two baseline recordings, and the intra- (IaPV) and inter-patient (IrPV) variabilities were estimated using a linear mixed model.[19] The reproducibility was evaluated using the intra-correlation coefficient (ICC). The interpretation of ICC is as follows: ICC <40% denotes poor reproducibility, 40% ≤ ICC < 75% characterizes good reproducibility and ICC ≥75% is associated with excellent reproducibility.[20]

P-values of ≤ 0.05 were considered statistically significant. The statistical analyses were done using SAS version 9.1.3 (SAS Institute, Cary, NC, USA).

Results

Study Population

The average age was similar in the placebo and moxifloxacin arms (27.5 ± 7.9 vs 26.5 ± 7.9 years; p = 0.38). The number of females in each arm was nine. No clinical information other than gender and age were released.

Moxifloxacin-Induced Repolarization Abnormalities

Table I contains the mean values and the standard deviations of the repolarization parameters measured in baseline ECGs from the placebo and the moxifloxacin arm, as well as the values for $\Delta_{moxi\text{-}baseline}$ and $\Delta_{placebo\text{-}baseline}$. Mean values of $\Delta\Delta$ for all repolarization parameters are also provided. The mean of QTc apex, QTc/QTcF, αL and $ERD_{30\%}$ were found to be statistically significantly different between the placebo group and the moxifloxacin group ($p < 0.05$). The differences for the amplitude of the T-wave and for αR were close to being significant ($p \leq 0.08$). Our analysis did not reveal any moxifloxacin-induced changes of the TpTe interval, the repolarization complexity ($\lambda 2/\lambda 1$) and the T-loop planarity ($\lambda 3$).

The results show that changes of repolarization segment on the surface ECG induced by moxifloxacin (and controlled for the placebo-effect) are affecting primarily the T-wave prior to the apex: αL is associated with a loss of −0.3 μV/ms ($p = 0.03$) and $ERD_{30\%}$ is prolonged by 7 ms ($p = 0.01$). Interestingly, this finding is supported by the values of $\Delta_{moxi\text{-}baseline}$ for QTc apex and $ERD_{30\%}$, which are prolonged by 4 ms. The repolarization signal following the apex of the T-wave is not significantly affected by the drug, and TpTe interval and LRD (30% and 70%) measurements remain essentially unchanged. The average decrease in αR does not reach the level of significance, and mainly reflects the changes in T-wave amplitude since the TpTe interval is not modified.

The vectocardiographic parameters did not show evidence of increased heterogeneity of repolarization in the complexity ($\lambda 2/\lambda 1$) or the planarity ($\lambda 3$) of the repolarization during drug therapy ($p = 0.9$).

Moxifloxacin-Induced Repolarization Abnormalities: Multivariate Analysis

In what follows, we built three binary logistic regression models to predict the presence of moxifloxacin based on three sets of clinical and repolarization parameters.

Table I. Baseline values, placebo- and moxifloxacin-induced changes of the repolarization signal[a]

Parameter	Placebo arm (n = 20)		Moxifloxacin arm (n = 20)		ΔΔ	p-Value
	baseline	Δplacebo-baseline	baseline	Δmoxi-baseline		
RR (ms)	944 ± 173	–59 ± 153	912 ± 154	–12 ± 175	–71	0.17
T amplitude (mV)	0.19 ± 0.08	0.015 ± 0.03	0.19 ± 0.07	–0.004 ± 0.05	–0.019	0.08
Heart-rate corrected QT measurements						
QTcB (ms)	418 ± 32	–3 ± 24	418 ± 32	7 ± 38	10	0.18
QTcF (ms)	406 ± 25	–7 ± 16	407 ± 25	6 ± 27	12	0.03
QTc apex (ms)	317 ± 21	–6 ± 11	323 ± 29	4 ± 17	10	0.02
QTc (ms)	398 ± 20	–8 ± 14	396 ± 28	2 ± 26	10	0.04
Heart–rate corrected repolarization measurements						
αL (μV/ms)	2.0 ± 0.8	0.2 ± 0.4	1.8 ± 0.6	–0.1 ± 0.54	–0.3	0.03
αR (μV/ms)	–3.2 ± 1.5	–0.4 ± 0.67	–2.8 ± 1.1	0.0 ± 0.92	0.4	0.06
Tsym	0.60 ± 0.19	0.03 ± 0.12	0.61 ± 0.14	0.02 ± 0.24	–0.01	0.70
TpTe (ms)	79 ± 13	–2 ± 7	77 ± 8	–2 ± 11	0	0.88
λ2/λ1	0.20 ± 0.08	–0.01 ± 0.09	0.20 ± 0.14	0.03 ± 0.18	0.04	0.90
λ3	0.05 ± 0.02	0.00 ± 0.03	0.05 ± 0.03	0.00 ± 0.03	0	0.90
$ERD_{30\%}$	37 ± 12	–3 ± 7	42 ± 11	4 ± 15	7	0.01
$LRD_{30\%}$	28 ± 6	–1 ± 5	27 ± 5	0 ± 6	1	0.40
$TRD_{30\%}$	76 ± 17	–4 ± 10	72 ± 18	2 ± 20	6	0.12
$ERD_{70\%}$	112 ± 34	–8 ± 21	111 ± 36	6 ± 44	14	0.10
$LRD_{70\%}$	59 ± 12	–2 ± 7	53 ± 11	–2 ± 14	0	0.51
$TRD_{70\%}$	178 ± 36	–9 ± 23	180 ± 43	4 ± 54	13	0.11

a All measurements (mean ± SD) are heart-rate corrected using their pooled formula but QTcB and QTcF are corrected using Bazett's and Fridericia's formula, respectively. p-Values are for testing for $\Delta_{moxi\text{-}baseline} \neq \Delta_{placebo\text{-}baseline}$.

αL = left slope of the T-wave; **αR** = right slope of the T-wave; ΔΔ = $\Delta_{moxi\text{-}baseline}$-$\Delta_{placebo\text{-}baseline}$; **λ2/λ1** = complexity of repolarization; **λ3** = planarity of T-loop; **ERD** = early repolarization duration; **LRD** = late repolarization duration; **TRD** = total repolarization duration; **QTc** = corrected QT interval; **Tsym** = symmetry of T-wave.

1. *QTc model:* First, we defined a clinical model including RR, gender, age and QTc interval as potential predictors. No other ECG parameters were included. Among the four predictors making up the clinical model, QTc interval was the only significant parameter. Its AIC was equal to 51.8, and the AUC of the associated ROC curve was equal to 0.71. This model, referred to as the 'QTc model', was used as a reference in subsequent analyses.

2. *Morphological model:* Second, we designed a model in which all repolarization parameters, QTc, QT apex, TpTe and morphological parameters were considered. Among models including a single parameter, the one based on $ERD_{30\%}$ was the first one to be selected, followed by $TRD_{30\%}$ and αR. The model based on $ERD_{30\%}$ had an AIC equal to 44.0, and provided a better fit to that of the QTc model when adjusted for the number of parameters. Its AUC was equal to 0.85, outperforming the QTc model in terms of discrimination. This model was based solely on repolarization parameters quantifying various morphological aspects of repolarization.

3. *Hybrid model:* Third, we considered additional candidate parameters in our logistic regression analyses to be added to the morphological model. The TpTe and QT apex intervals were selected. The AIC corresponding to this model was lower than for the previous models (38.4). The AUC was equal to 0.91. A second hybrid model, forcing on QTc but excluding TpTe and QT apex, was also tested. The resulting model had a larger AIC (42.3) than the first hybrid model; its AUC was slightly lower than that of the first hybrid model (0.87).

Figure 2 shows the ROC curves for these three models. The point 'optimizing' both sensitivity and specificity is indicated on the figure for each model. In the hybrid model, sensitivity and specificity were

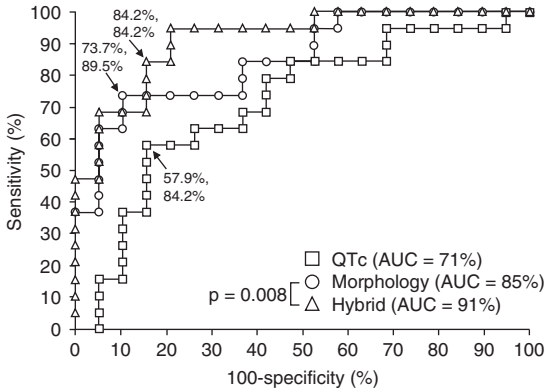

Fig. 2. Receiver operating characteristic (ROC) curves from binary logistic models for discriminating ECGs on moxifloxacin from ECGs on placebo. The ROC curves computed from three binary logistic models were used to classify the effects of moxifloxacin vs placebo. The arrows indicate the location of the points where the sum of sensitivity and specificity, respectively, are maximized for each model. **AUC** = area under concentration-time curve.

both equal to 84.2%. The morphological model resulted in a sensitivity of 73.7% and a specificity of 89.5%. The QTc model yielded a sensitivity of 57.9% and a specificity of 84.2%. The hybrid model improved the discrimination between the ECG tracings by 20% in terms of the AUC, and a gain of 26.3% for the sensitivity.

Based on the LR test, the hybrid model was found to be significantly different from the morphological models ($p = 0.008$); thus, providing improvement in terms of predictive power. In addition, we compared the QTc model with the morphological model after adding QTc in this last one in order to have two nested models (see Statistical Section for the LR test requirement of nested models). The p-value comparing the ROC curves of these two nested models was equal to 0.001, demonstrating that the morphological parameters bring independent and significant information for the discrimination of ECGs on moxifloxacin or placebo.

This analysis suggests that moxifloxacin is primarily delaying specific portions of the T-wave located prior to the apex of the T-wave, and this prolongation is reflected in the overall prolongation of the QT interval and QT apex interval but not in the TpTe interval. The delay is associated with morphological changes linked to a reduction of the amplitude of the T-wave. A linear regression analysis between $ERD_{30\%}$ and QTc revealed a significant relationship between the two parameters for $\Delta_{placebo\text{-}baseline}$ ($R^2 = 42.2\%$; $p = 0.003$), but this relationship vanished for $\Delta_{moxi\text{-}baseline}$ ($R^2 = 0.0\%$; $p = 0.9$). This result emphasizes the idea that $ERD_{30\%}$ is combining information about prolongation and other morphological changes contributing to its increased ability to detect the presence of moxifloxacin. Figure 3 provides simulated tracings of the averaged representative repolarization segment on and off moxifloxacin (bold and dotted tracings, respectively) based on the results reported in table I.

Comparison of Repolarization Parameters: Sensitivity Analysis

Table II reports the values of IaPV/IrPV and reproducibility (based on the ICC) of repolarization parameters. We investigated short-term (between

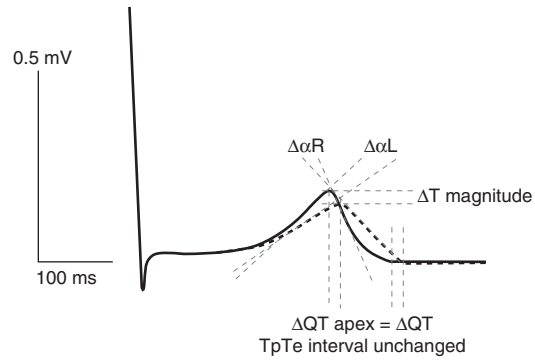

Fig. 3. Moxifloxacin-induced changes of the T-wave on the scalar ECG. Two superimposed simulated ECG signals based on the values and drug-induced changes of the repolarization parameters reported in table I. The ECG tracings with the shorter QT interval is constructed based on the baseline values reported in table I (QT apex, QT, αR, αL and T amplitude), and it represents the averaged T-wave morphology in healthy individuals. The dotted tracing is constructed based on the Δ values of these parameters reported in table I. We observed that TpTe interval was not changed by moxifloxacin (ΔQT apex = ΔQT), but the downslope of the T-wave was decreased, leading to morphological changes in the second part of the T-wave. The moxifloxacin-induced delay of repolarization is concentrated in the interval just prior to the apex of the T-wave where the slope of the T-wave is most changed by both the decrease of T-wave amplitude and slow movement of the repolarization front. α**L** = left slope of the T-wave; α**R** = right slope of the T-wave.

Table II. Sensitivity analysis: short- and long-term reproducibility and intra- and inter-patient variability of repolarization parameters

Parameters	Short-term			Long-term		
	IrPV	IaPV	Rep. (%)	IrPV	IaPV	Rep. (%)
QTc apex (ms)	17.9	10.0	76	17.0	12.1	67
QTc (ms)	17.0	12.0	68	12.1	20.7	26[a]
TpTe (ms)	9.5	9.5	50	7.0	14.8	18[a]
$LRD_{30\%}$ (ms)	4.9	4.0	60	5.4	4.0	64
$ERD_{30\%}$ (ms)	10.5	5.6	77	7.3	8.4	43
T amplitude (mV)	0.05	0.03	74	0.04	0.03	63
αL (μV/ms)	0.52	0.35	69	0.46	0.41	55
αR (μV/ms)	0.87	0.58	69	0.71	0.62	57
Complexity (λ2/λ1)	0.03	0.07	14[a]	0.07	0.06	57
Planarity (λ3)	0.01	0.02	33[a]	0.02	0.01	82

a Values indicate the repolarization parameters with poor level of reproducibility (<40%).

αL = left slope of the T-wave; **αR** = right slope of the T-wave; **λ2/λ1** = complexity of repolarization; **λ3** = planarity of T-loop; **ERD** = early repolarization duration; **IaPV** = intra-patient variability; **IrPV** = inter-patient variability; **LRD** = late repolarization duration; **QTc** corrected QT interval; **Rep.** = reproducibility based on intra-correlation coefficient value.

hours, same day) and long-term (between days, same hour) variability and reproducibility of the repolarization parameters. We compared baseline measurements done within the same day and measurements from ECGs recorded at day 1 versus day 2. The $ERD_{30\%}$ and QT apex parameters have an excellent short-term reproducibility with ICC values of 0.77 and 0.76, respectively. QT, TpTe, LRD, T-wave amplitude, αR and αL slopes have good short-term reproducibility with ICC values ranging from 0.50 to 0.74. The complexity and planarity of the repolarization loop were associated with the lowest ICC values (0.14 and 0.33, respectively) revealing their poor short-term reproducibility.

The IaPV and IrPV should not be compared between parameters since they have either different units or scales. When comparing intra- to inter-patients short-term variability by parameters, all the parameters have lower IaPV than IrPV ($ERD_{30\%}$: IaPV = 5.6 ms, IrPV = 10.5 ms; QT apex: IaPV = 10.0 ms, IrPV = 17.9 ms; QT: IaPV = 12.0 ms, IrPV = 17.0 ms) except the complexity (IaPV = 0.07, IrPV = 0.03 ms) and the planarity (IaPV = 0.02, IrPV = 0.01 ms) parameters.

The long-term variability and reproducibility of the parameters were different from the short-term ones. On average amongst all investigated repolarization parameters, the short-term reproducibility is slightly higher than the long-term (59% vs 53%). More interestingly the QT and TpTe intervals reveal poor long-term reproducibility mainly driven by larger intra-patient variability. There were significantly different changes in heart rate between the two sets of ECGs considered for long-term sensitivity analysis (moxifloxacin vs placebo arms): 21 ± 140 versus 178 ± 148 ms ($p = 0.007$). Whereas for the short-term variability, the difference in heart rate changes were: -124 ± 140 versus -31 ± 160 ms ($p = 0.14$). After correction for heart rate, none of the repolarization parameters used in our models showed significant changes between baseline periods. Nevertheless, one would note that Bazett's and Fridericia's formulae have correction errors that depend on heart rate values and thus comparing groups with very different heart rates might not be appropriate. The $ERD_{30\%}$ and $LRD_{30\%}$ parameters show also lower long-term reproducibility, but they remain in a good range (>40%).

The direct impact of having a more stable measurement of repolarization delay than QT/QTc is to be able to detect a drug effect with fewer individuals at the same level of statistical power. Figure 4 illustrates this point by showing the power curves for QTc to detect a 5-ms prolongation and for $ERD_{30\%}$ to detect several effect sizes (3.5-, 4- and 5-ms prolongation) in ECGs of individuals receiving placebo versus patients receiving moxifloxacin in a parallel-designed study. All parameters were heart

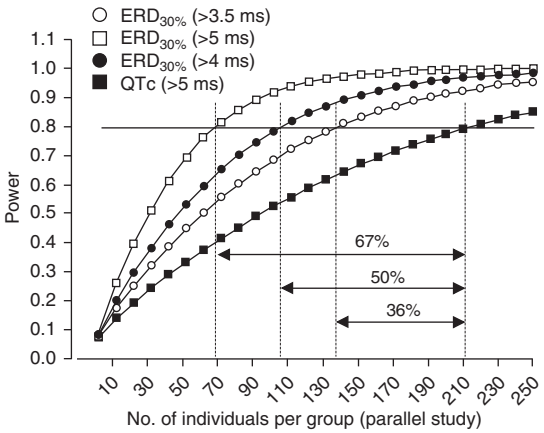

Fig. 4. Power and number of subjects to consider in each arm of a parallel study to detect 5 ms QTc differences and to detect several effect sizes for early repolarization duration $(ERD)_{30\%}$. The four curves represent the power level as a function of the number of subjects in each arm of a parallel study to detect effect sizes of 3.5, 4 and 5 ms for $ERD_{30\%}$, and of 5 ms for QTc prolongation based on a one-sided t-test at the 5% level of significance. **QTc** = corrected QT interval.

rate corrected using the same technique. $ERD_{30\%}$ was fully computerized while QTc was measured using a semi-automatic technique. The values were based on a one-sided t-test at the 5% level of significance. For an 80% powered study, 5 ms prolongation is detected with 69 individuals per arm when using $ERD_{30\%}$, and 211 individuals when using QTc interval; thus, resulting in a 67% reduction of the sample size of thorough QT studies. Because the relevant effect size for $ERD_{30\%}$ is unknown we also investigated smaller effects: 4 ms and 3.5 ms. As shown in figure 3, a trial based on $ERD_{30\%}$ would require fewer subjects than a trial based on QTc.

Discussion

We developed a set of indices for measuring morphological features of the repolarization segment on the surface ECGs. These measurements aim at providing quantitative information about specific portions of the repolarization signal. Digital quantitative electrocardiography allows the use of digital signal processing tools to quantify morphological aspects of the repolarization segment that are difficult to assess visually on ECG tracings. Most of the findings relate to specific portions of the QT interval.

The changes in ion kinetics of myocardial cells affect the duration and the overall shape of the action potential of the myocyte,[1] with most emphasis to date on the prolongation of the action potential and its resulting expression on the surface ECG by prolongation of the QT interval. We suggest that moxifloxacin-induced changes of the surface ECG are 2-fold: an unevenly-distributed delay of the repolarization process (primarily occurring prior to the apex of the T-wave) and changes in the morphology of the T-wave (amplitude decrease and reduction of up- and down-slope of T-wave). Consequently, a parameter depending on both the duration and the amplitude of the signal (such as the $ERD_{30\%}$ parameter) can outperform QTc for the detection of the presence of moxifloxacin.

We hypothesize that the changes in ion kinetics in the cells throughout the ventricular myocardium are reflected in the morphology of the repolarization segment. In the hereditary form of the long QT syndrome, an increased number of observational studies have confirmed the use of repolarization morphology in the identification of the type of congenital LQTS.[16,21,22] In this study, we provide a multivariate model that significantly improves the detection of the drug moxifloxacin, known to be an I_{Kr}-inhibitor, based on T-wave morphology measurements.

Morphological Analysis of T-Wave in the Congenital and Acquired Long QT Syndrome

QT prolongation is recognized as an imperfect surrogate marker of drug-induced cardiotoxicity. Some drugs are known to be associated with QT prolongation without inducing TdP.[23] However, there is an accepted and widely-held belief that the I_{Kr}-inhibition is a common ionic mechanism of drug-associated cardiotoxicity related to a delayed repolarization process. If the tested drug interacts with more than one ion current then it is likely that this interaction may mitigate or exacerbate the effect on the ventricular repolarization process. A large

QT interval prolongation effect (>500 ms) is a very good risk marker, but previous experience has shown that the predictive value of shorter QT prolongation is much weaker.[11] Consequently, an interesting challenge of modern digital electrocardiography is to determine whether an electrocardiographic test could identify specific abnormalities associated with an increased risk for arrhythmic events.

In prior investigations from our group, we showed that the morphology of the T-wave may be combined with QT interval prolongation for improving the detection of a *KCNH2* mutation in people with the hereditary LQTS and a borderline QT prolongation.[16] In the acquired form of LQTS, we identified morphological changes in addition to QT prolongation.[13] The current study reinforces these prior results on the role of repolarization morphology in identifying the presence of a drug that inhibits I_{Kr}-kinetics in myocardial cells.

In a study involving erythromycin, an antibacterial drug with dose-dependent I_{Kr}-inhibition properties, Antzelevitch et al.[24] demonstrated in a canine model at higher dose than those measured in human plasma (10–100 μg/mL) that "a prominent dispersion of repolarization across the ventricular wall is setting the stage for induction of TdP-like tachyarrhythmias displaying characteristics typical of reentry". This group showed that QT prolongation is often associated with an increased heterogeneity of cardiac repolarization across the cardiac ventricle wall due to a prolongation of the action potential of the cells in the mid-myocardium (so-called M cells), but not in the epicardial and endocardial cells.

In more recent work from Chen et al.,[25] hyperdoses of moxifloxacin (~18-fold above the typical unbound maximum-concentration exposure in clinical settings) were associated with an increased risk of inducing TdP. This group also reported a concentration-dependent prolongation of the QT interval and of the TpTe interval (potential surrogate marker of transmural repolarization dispersion). In this last report, the lack of TdP on moxifloxacin is attributed to "its predicable peak concentration profile and other dose-limiting effects" emphasizing that it is unlikely to find large variation of plasma concentration of the drug in humans because of the occurrence of other adverse effects such as dizziness, headache and diarrhoea limiting the possibility of finding high-level drug exposure.

In the general population, it is accepted that the risk associated with a normal dose of moxifloxacin in human is extremely low. In our results, based on standard 12-lead ECGs, the TpTe interval was not very strongly associated with drug-induced changes when using univariate analysis whereas QT and QT apex intervals were significantly prolonged by the drug.

The parameter $ERD_{30\%}$ measured abnormalities of ECGs prior to the apex of the T-wave. It is noteworthy that the left slope of the T-wave was also showing significant changes associated with moxifloxacin, as the same slope was found to be relevant in the identification of LQTS carrier of a *KCNH2* mutation.[16] Of note, a recognized trigger of TdP is the occurrence of an R-on-T extrasystole or phase 2 early after depolarization. The phase 2 potentials very likely contribute to the formation of the signal prior to the apex of the T-wave on the surface ECGs. It is during this very same short time interval of the T-wave that an external chest wall blow triggers arrhythmic events (commotio cordis), i.e. between 10 and 30 ms before T-peak.[26]

Limitations of the Study

Developing a new more-stable measurement than QT interval prolongation for the detection of an abnormal repolarization process linked to I_{Kr}-abnormalities is important, but remains a very preliminary step. The role of these new ECG measurements in drug-safety assessment requires association of their relationship with cardiac events. Reaching such a goal will depend on a better understanding of the underlying mechanisms leading the I_{Kr}-inhibition to the occurrence of TdP.

Such studies will require access to ECG datasets from individuals receiving torsadogenic drugs. As far as the authors know, such ECG databases are lacking, and when they exist they encompass rather low-quality ECG tracings (paper tracings). The use

of ECG datasets from the International LQTS Registry should be very useful because it contains a rather large set of endpoints such as syncope, aborted cardiac arrest and sudden cardiac death.[27,28] The arrhythmogenic mechanism in the hereditary LQTS, especially LQT type-2, is likely to be similar to the one found in drug-induced arrhythmias and thus, the hereditary LQTS might be a very good paradigm.

Our study provides a first step investigation into T-wave morphological changes associated with the weak I_{Kr}-inhibitor, moxifloxacin in comparison to QT interval prolongation when measured from lead II uniquely. We plan to validate the model developed in this work on a larger set of tracings from the FDA ECG Warehouse and this future study will help us to address several limitations of this pilot study, such as: understanding the contribution of other leads than lead II, assessing the concentration-dependent effect of moxifloxacin on our new ECG measurements and validating our novel ECG markers on data recorded in cross-over designed studies to eliminate potential differences in drug-sensitivity amongst study groups.

Conclusion

Repolarization morphology is significantly altered by moxifloxacin, a drug inhibiting the I_{Kr} current. The computerized ECG technique provides a novel method of quantifying morphological changes of repolarization segment. In this preliminary study, our new parameters reflecting the morphology of the T-wave provided better identification of the presence of moxifloxacin-induced I_{Kr} blockade in healthy individuals than the QTc interval.

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