A Novel Method for Poincaré Plot Shape Quantification Demonstrates Cardiac Tissue Repolarization Inhomogeneities Induced by Drugs

S Mensing[1], J Limberis[2], G Gintant[2], A Safer[3]

[1]Abbott GmbH & Co KG, Ludwigshafen, Germany
[2]Abbott, Dept. Integrative Pharmacology, Abbott Park IL, USA
[3]Drug Safety Consultant, Weisenheim am Sand, Germany

Abstract

According to ICH S7B Guidelines evolving drug candidates need to be evaluated for effects on cardiac repolarization. An important model is the evaluation of action potential duration prolongation in vitro.

The use of sinus arrhythmia pacing protocols (mimicking sinus arrhythmia in vivo) generates a cyclic structure from the repolarization duration. We use Poincaré Plot derived metrics to quantify (i) central AP duration, (ii) dynamical range of AP duration and (iii) principal shape of APD trajectories expressing repolarization variability.

A proof of concept study (N=3) was performed evaluating the effect of the reference drug moxifloxacin using Purkinje fibers and papillary muscles with standard microelectrode techniques. Poincaré shape metrics under moxifloxacin treatment resulted in distinctly different responses between the tissue types. We conclude that the proposed method has potential for an improved quantification of repolarization variability.

1. Introduction

The preclinical assessment of pro-arrhythmic potential of pharmaceutical substances is frequently performed using isolated cardiac tissues usually stimulated at constant rates or sometimes employing different basic cycle lengths in a stepwise protocol. This fixed pacing interval stimulation protocol applied to in vitro preparations is very far from physiological conditions found in living animals. For example, conscious dogs under resting conditions show a distinct pattern of respiratory driven sinus arrhythmia. Variations in the heart rate of a beagle dog vary between 400 and 1200 to 1400 milliseconds throughout a single respiration cycle, at a respiration rate between 6 and 10. Thus, we applied a sinus arrhythmia stimulation protocol (derived from telemetry in conscious beagle dogs under resting conditions) to cardiac tissues in vitro to mimick sinus arrhythmia.

Characterizing rate-dependent changes in the action potential duration (APD) in the absence of constant rate

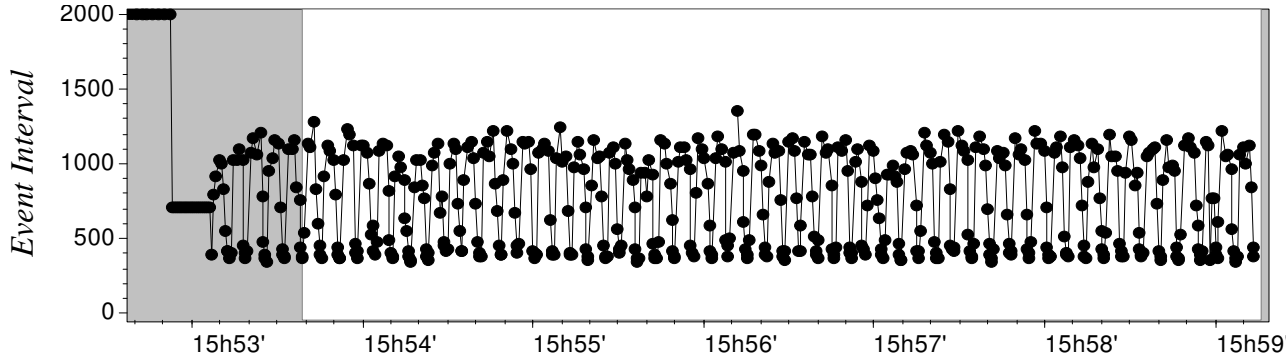

Figure 1: The inter-beat distance of the sinus arrhythmia stimulation protocol.
The constant pacing is accelerated before the sinus arrhythmia protocol is applied. After the sinus arrhythmia pacing phase, a linear pacing is applied until the next recording phase.

stimulation can be assessed for bins of different heart rates. This binned evaluation can provide valuable insights into the effect of the heart rate fluctuations on the influence of pharmaceutical drug substances on the dynamics of repolarization. Nonetheless it is desired to quantify the changes in the dynamical behavior of repolarization.

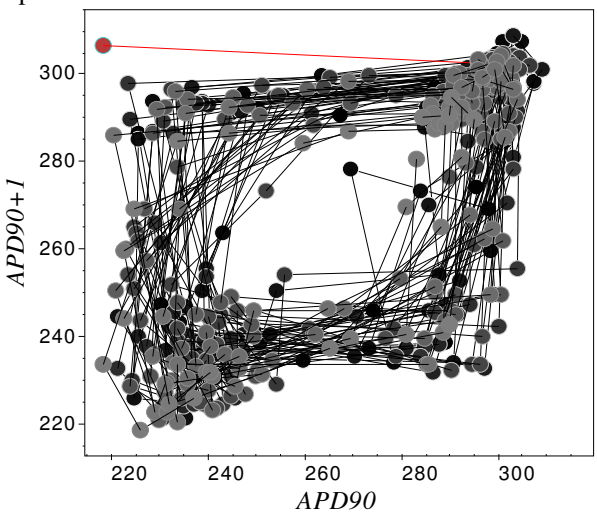

Figure 2: Typical Poincaré plot for APD90.

Poincaré plots of the APD values graphically summarize the complex, nonlinear dynamics of repolarization driven by the heart rate changes. We are plotting the repolarization duration returning to a 90% threshold of the maximum depolarization, (APD_{90}) from (i)-th beat vs. the next stimulus, APD_{90} (i+1).

These plots are helpful to detect drug changes in the repolarization trajectories. Typically, the picture exhibits the cyclic distorted ring-like structures of the response, which resist any meaningful classical statistics; not even the least conditions of distribution unimodality or symmetry are met. In order to quantify the properties, we need to characterize the ring-form for an average location, extension of the structure as a measure of variability, and to allow a description of the ring trajectory that can describe the average trajectory location in relation to the position of the centric location of the structure. Moreover, as the trajectories show in parts smooth changes, and in other parts twists in the trajectory direction, we need a flexible method. Since extreme observations are quite common, the method has to prove robust against a subset of extreme observations. To be useful for shape assessment, we need measures to characterize a space of percentiles, or build belief limits.

2. Methods

Purkinje fibers with attached portions of ventricular endocardium and papillary muscle were isolated and mounted in superfusion chambers (37° C). Action potentials were simultaneously recorded using standard microelectrode techniques, providing stable, high fidelity transmembrane action potentials. Preparations were stimulated using bipolar electrodes; bipolar stimulation waveforms (typically <2 msec duration) were delivered from a Frederick Haer Pulsar 6bp a/s stimulator (FHC Inc, Bowdoin ME) triggered by custom programmable software that followed the canine sinus arrhythmia protocol. Signals were recorded using pClamp (version 8.2, Molecular Devices Corp, Sunnyvale CA); action potentials were analyzed using ABBIOS workbench system software [2]. Derived values were checked visually and by cross-referencing to various other monitored electrophysiologic parameters (resting membrane potential, action potential overshoot) for consistency. Prior studies had demonstrated comparable APD_{90} values obtained automatically using ABBIOS. The canine sinus arrhythmia consisted of a sequence of 360 beats (43 respiratory cycles, approximately 4.5 min duration) derived from R-R intervals of an EKG recorded from a telemeterized, resting conscious Beagle dog. A slow-pace starting sequence with 2000 ms basic cycle length (BCL) was used, followed by a constant BCL adaption to 765 ms (the median value of the sinus arrhythmia protocol sequence) prior to start of the sinus arrhythmia protocol.

Responses of Purkinje fibers to moxifloxacin were recorded successively under three different treatment conditions: control, 3 µg/ml moxifloxacin (corresponding to the average maximal total plasma concentration four hours after oral ingestion of 400 mg moxifloxacin in adult humans), and 30 µg/ml moxifloxacin (equivalent to ten times the average maximum total plasma concentration). The duration of measurement phase for each treatment was approximately 6 minutes, with 30 minutes equilibration periods between measurements.

The Poincaré plot (Figure 2) shows the cyclic structure of the APD responses (termed the repolarization trajectory). In order to estimate a medial trajectory and belief limits of the medial trajectory, the Algorithm for Pre-/Clinical Analysis of Repolarization Trajectories (APART) has been developed. A MATLAB® based program has been provided that takes charge of the calculations, providing the subsequently described measures. A preprocessing step substitutes missing values, and removes trends in the time series by interpolating gaps in the time series using an autoregressive moving average (ARMA) model. If the gaps are greater than 4 missing values, the disconnected time segments are connected by matching the end of the first fragment with the end of the second one. Some values of the second segment may be dropped in order to maximize the correlation of the first and second segment. Trends in the time series are removed by applying a bidirectional moving average filter.

The idea of APART is to estimate a medial trajectory

that is embedded by the cyclic structure in the Poincaré plot. From this medial trajectory orthogonal lines (or hyperplanes in the multi-dimensional extension of this method) are constructed. Using the intersection points of these lines with the repolarization trajectories percentiles or belief limits may be constructed.

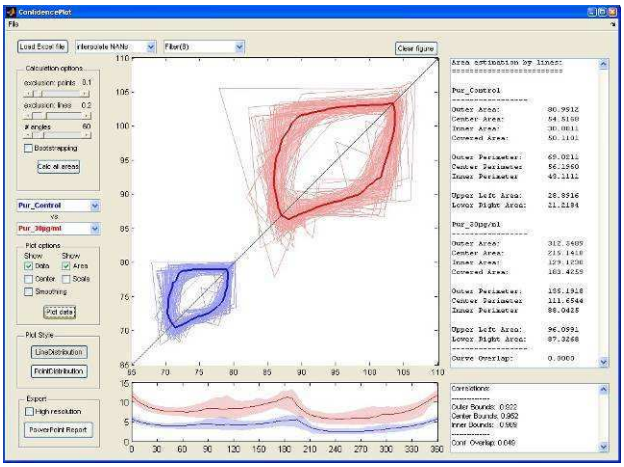

Figure 3: Screenshot of the APART GUI developed using MATLAB® (http://www.mathworks.com), Version 2007b.

It has been found that the algorithm can be improved by alterations to the algorithm, which produced robust results for the given preclinical data. Rather than estimating the medial trajectory by some curve fitting algorithms, the medial trajectory and its percentiles or belief limits are estimated in one step.

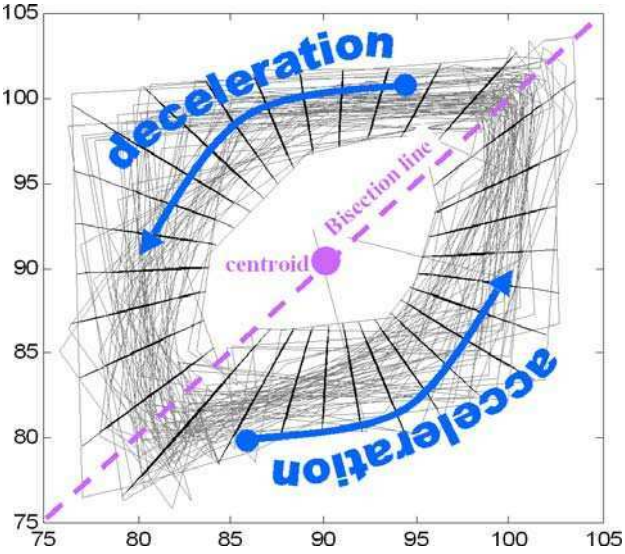

Figure 4: Illustration of the determination of the intersection points. The intersections of the rays (black) with the repolarization trajectory (grey) are shown.

In order to estimate the medial trajectory, a centroid of the repolarization trajectories needs to be found. The following definition of the centroid has been found to be sufficient:

$$C = \left(\frac{P_{10}(x) + P_{90}(x)}{2}, \frac{P_{10}(y) + P_{90}(y)}{2} \right)$$

where P_k denotes the k-th percentile, x and y denotes the two-dimensional coordinates of the points in the Poincaré plot. Thus the centroid is defined as the mean of the 10th and 90th percentile of the coordinates of the points in the Poincaré plot.

Starting from the centroid a specified number of equiangular rays are constructed. The intersection points of these rays with the repolarization trajectories are calculated. These points may be used to construct the medial trajectory (connection of the medians of each rays intersection points) and the percentiles or belief limits.

The belief limits are constructed using bootstrapping since no assumptions on the distribution of the intersection points are appropriate. Thus B sets of sampling points are drawn randomly from the sampling points with repetition. For each of the B sets, the median is calculated and of these B medians the 5th and 95th percentile correspond to an estimate of the 90% belief limit of the medial trajectory.

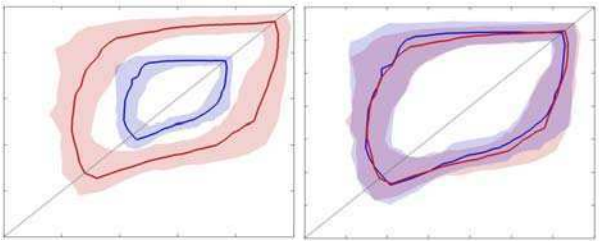

Figure 5: Left: medial trajectories for the APD90 trajectory of a Purkinje fiber under 0µg/ml (blue) and 30µg/ml (red) moxifloxacin. The centroids for the trajectories are set to (0,0).
Right: the same medial trajectories centered and scaled. The original trajectories are shown in figure 3.

The trajectories can be analyzed on three different levels, as the central APD is given by the location of the centroid. The range of the APD trajectories can be compared by computing the perimeter of the medial trajectory and its belief limits. A visual representation of the range of the APD trajectories can be achieved by plotting the trajectories after the centroid has been set to (0,0). This is illustrated in figure 5.

In order to compare the principal shapes of the trajectories the centroid is moved to (0, 0) and the medial trajectory is adjusted to have a constant length (e.g. 1) for each repolarization trajectory. For each of these standardized medial trajectories the amount of the trajectory part below the bisection can be calculated. The amount above

the bisection line yields a measure for the time spent in deceleration phases and the fraction below the line represents acceleration phases.

The medial trajectory is obtained by the intersection of equiangular rays with the repolarization trajectories; it can be visualized as a unfolded angular graph (cmp. Figure 6). For these series treatment differences and their belief intervals (analogous to confidence limits) can be calculated in order to describe and compare the principal shape of the trajectories with statistical procedures.

Another approach is the fraction of the curves overlapping (cmp. Figure 6 with strong overlap). Given a measure of the agreement of the dynamical range and shape of the trajectories. APART has been implemented in MATLAB® and a graphical user interface has been constructed (cmp. figure 3). The GUI allows the configuration of all mentioned properties of APART and automatically generates measurements on the medial trajectories. An automated report generation is projected.

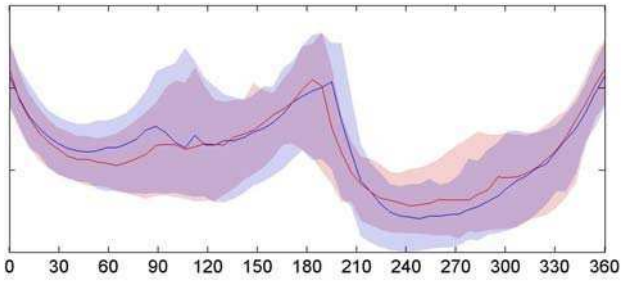

Figure 6: Graphical representation for the distance of the medial trajectories to the centroid.

3. Results

In comparison of the log perimeter size, no change for papillary muscle can be detected, while moxifloxacin elicits concentration-dependent increases in the perimeter derived from Purkinje fibers.

The median perimeter for the papillary muscles was changed to 93% (SD: 2) and 105% (SD: 19) under 3µg/ml and 30µg/ml drug relative to control. The perimeter of the medial curve for the Purkinje fiber trajectory increased to 114% (SD: 5) and 182% (SD: 16) under 3µg/ml and 30µg/ml drug relative to the perimeter of control conditions.

Shapes of trajectories, assessed by the correlation of the distance to the centroid, were not changed significantly by moxifloxacin.

4. Discussion and conclusions

The Algorithm for Pre-/Clinical Analysis of Repolarization Trajectories (APART) has been developed to quantify changes in the repolarization dynamics of tissues stimulated using physiologic (non-constant) patterns. A graphical user interface has been implemented in MATLAB® to facilitate the application of the method.

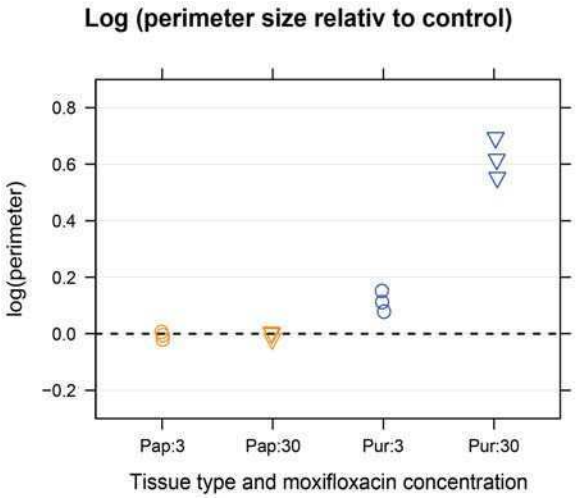

Figure 7: Treatment and tissue effects of the log perimeter of the medial trajectory relative to placebo.

The algorithm allows the assessment of changes in mean AP duration, dynamic range of the AP durations and changes in the principal shape of APD trajectories. Additional studies employing APART to characterize drug-induced changes in repolarization dynamics are necessary to determine the utility of this approach in defining pro-arrhythmic risk of evolving drug candidates.

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References

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Address for correspondence

Abbott GmbH & Co. KG
Sven Mensing
Knollstr. 50
67061 Ludwigshafen, Germany
(sven.mensing@abbott.com)