ABSOLUTE BEAT-TO-BEAT VARIABILITY AND INSTABILITY PARAMETERS OF ECG INTERVALS PREDICT ISCHEMIA-INDUCED VENTRICULAR FIBRILLATION

Ph.D. thesis

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LIST OF PUBLICATIONS

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   New in vitro proarrhythmia model that is based on reduced repolarization reserve
   Interventional Medicine and Applied Science 2011; 3: 3 pp. 157-157., 1 p.

2. **Sarusi, A**; Forster, T; Curtis, MJ; Farkas, A
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3. **Sarusi, A**; Forster, T; Curtis, MJ; Farkas, A
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   Cardiologia Hungarica 2011; 41: Suppl. F pp. F42-F43, 1 p.

4. **Sarusi, A**; Farkas, A; Orosz, Sz; Forster, T; Varró, A; Farkas, A
   Screening proarrhythmia - validation of new biomarkers and a new in vitro model based on reduced repolarization reserve
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1. INTRODUCTION

The primary cause of sudden cardiac death is ventricular fibrillation (VF) triggered by acute ischemia, the prediction of which remains a significant challenge to date. The aim of this study was to analyze whether a previously validated Torsades de Pointes (TdP) prediction method developed by our research group can be applied to predict VF occurring during early ischemia.

Previous studies have demonstrated that the role of ventricular repolarization variability in predicting Torsades de Pointes (TdP) risk is limited when based on analyses of arrhythmia-free ECG data. In contrast, measuring the beat-to-beat variability and instability (BVI) of ECG intervals independently of rhythm has proven to be an accurate and reliable predictive method. To clearly distinguish these parameters from rhythm-dependent BVI parameters derived under stable sinus rhythm and reported by other researchers (sinus BVI), we introduced the term "absolute" to refer to BVI parameters derived independently of rhythm.

The maintenance of TdP is based on functional re-entry circuits facilitated by the spatial dispersion of ventricular repolarization. Absolute BVI parameters quantify electrical instability, thereby characterizing the substrate for re-entry arrhythmias, such as TdP. Although ischemia-induced VF and TdP differ in many respects, they share the common feature of functional re-entry. In the absence of a reliable predictive method for ischemia-induced VF, we investigated whether absolute BVI parameters can predict phase I ischemia-induced VF in isolated rat hearts, thereby assessing their applicability beyond the context of TdP.

Isolated Langendorff-perfused rat hearts serve as a validated model for studying the mechanisms of ischemia-induced VF. Since the substrate for VF, electrical inhomogeneity, is similar across mammalian species, this model is presumed to be suitable for testing absolute BVI parameters as well. By reanalyzing data from previous studies using a novel method, this study demonstrated that absolute BVI parameters predicted ischemia-induced VF, whereas sinus BVI parameters were not effective for this purpose.

2. AIMS OF THE STUDY

The objectives of the present study were as follows:

I. To investigate whether the novel arrhythmia biomarkers, the absolute BVI parameters of ECG intervals, can predict the occurrence of phase I ischemia-induced VF in isolated rat hearts.

II. To assess whether the rhythm-independent mean values of ECG intervals, measured even during arrhythmias (absolute mean ECG intervals), can predict phase I ischemia-induced VF in isolated rat hearts.

III. To evaluate whether the mean ECG intervals and derived BVI parameters, measured during periods without sinus rhythm, are suitable for predicting phase I ischemia-induced VF in isolated rat hearts.

IV. To examine the frequency of ventricular premature beats, including 'R-on-T' arrhythmic beats, in terms of their ability to predict phase I ischemia-induced VF in isolated rat hearts.

V. To assess the diversity of ventricular premature beats in terms of their contribution to the development of phase I ischemia-induced VF in isolated rat hearts.

VI. To identify the ECG parameters with the best predictive power, sensitivity, and specificity for forecasting the occurrence of ischemia-induced VF in isolated rat hearts.

3. METHODS

3.1 Animals and General Experimental Methods

In a previous study conducted on isolated Langendorff-perfused rat hearts (n=24), two independent, drug-free control groups followed the same protocol, which included 30 minutes of localized ischemia. Since no significant differences were observed in the baseline data between the two control groups, they were combined into a single group. In this study, hearts from male Wistar rats were excised under anesthesia and perfused with Krebs solution. For ECG recording, a unipolar electrode was placed at the center of the ischemic region, while another electrode was attached to the aorta. Regional ischemia was induced by occlusion of the left main coronary artery. Continuous ECG recordings were obtained during the ischemic period.

3.2 Experimental protocol

Following a 10-minute perfusion with Krebs solution, the left main coronary artery was occluded for 30 minutes in all 24 hearts. The experimental protocol consisted of two main phases: the pre-ischemic "baseline" period and the post-ischemic phase, during which the development of VF was analyzed.

3.3 Groups

During the first 15 minutes of ischemia, hearts that exhibited VF were categorized into the "VF+" group, while those without VF were classified into the "VF-" group. By comparing the data between these two groups, we identified the ECG parameters with predictive value for the development of VF.

3.4 Measurement of the ECG intervals

The measurement of ECG intervals was performed at the end of the experiment during playback of the recordings, with the assistance of an experienced expert. To eliminate observer bias, the measurements were conducted in a blinded manner. The RR, QR, RT, QT, and DI intervals were manually determined using marker lines placed on the ECG traces. The QT wave of the surface ECG reflects the global indicator of ventricular action potential duration (APD), while the JT interval represents ventricular repolarization, measured from the J-point (the transition between the QRS complex and the ST segment) to the end of the T wave. In small rodents, such as rats, the first phase of repolarization merges with depolarization due to the Ito K+ current. Consequently, the QT interval, which includes depolarization, is not ideal for describing repolarization. Therefore, the RT interval, measured from the R peak to the end of the T wave, was also determined as it provides a more accurate representation of true repolarization.

During ventricular arrhythmias, the T wave often overlaps with the QRT wave of the subsequent beat, a phenomenon referred to as an "R on T" ventricular premature beat (VPB). In such cases, repolarization was determined through extrapolation: the end of the T wave was extended to the isoelectric line beneath the QRT complex, and this was designated as QTx. Ventricular DI was defined as the time interval from the end of the T wave to the beginning of the next QRT complex. However, if the T wave was truncated by the QRT complex, the DI value was set to 0.000001 ms.

3.5. Obtaining absolute and sinus BVI parameters

The RR, QR, RT, QT, QTx, and DI intervals of 40 consecutive ventricular beats were measured, and the following BVI parameters for each interval were calculated using specific formulas:

- **RMSSD and SDSD**: Root mean square and standard deviation of successive differences.
- **STV and LTV**: Average perpendicular (STV) and parallel (LTV) distances from the line of identity in the Poincaré plot.
- **TI, LTI, STI**: Parameters calculated using complex mathematical methods.
- **Instability**: Defined as the interquartile range difference.

BVI parameters were classified as **sinus** if derived from 40 consecutive QRT intervals during sinus rhythm, and **absolute** if measured independently of rhythm at predefined time points.

- **Sinus BVI**: Calculated from a sample taken during the last arrhythmia-free period—immediately prior to VF in the VF+ group, and at the 15th minute of ischemia in the VF- group.
- **Absolute BVI**: Determined in the last minute before coronary occlusion, at the 7th minute of ischemia, and immediately prior to VF in the VF+ group or at the corresponding time point in the VF- group.

The frequency of arrhythmic and "R on T" beats was expressed as a percentage relative to the 40 analyzed beats.

3.6. Morphological characterization of the arrhythmic beats before VF

For the analysis of arrhythmic beats, the last 40 beats in the VF+ and VF- groups were examined. The R-wave amplitude, as well as the QR, QT, DI, and coupling intervals, were measured. The data were compared between the two groups to identify morphological characteristics that may contribute to the development of VF.

3.7. Statistics

Data were expressed as mean ± standard error of the mean (SEM). Comparisons between groups were performed using the Mann–Whitney test, while the incidence of VF was evaluated using Fisher's exact test. The predictive value of ECG parameters was assessed through receiver operating characteristic (ROC) curve analysis, with an area under the curve (AUC) value above 0.8 considered a valid predictor. The sensitivity and specificity of the best predictors were determined based on the Youden index.

4. RESULTS

4.1. Comparison between the ‘VF+’ and ‘VF-’ groups

4.1.1. Absolute mean ECG intervals

Before coronary occlusion, no differences were observed in the mean RR, QR, RT, QT, QTx, and DI intervals between the 'VF+' and 'VF-' groups. At the 7th minute of ischemia, the QR interval was longer in the 'VF+' group. At the "pre-VF" time point, the RR, QT, and RT intervals were shorter in the 'VF+' group, indicating a higher frequency of 'R-on-T' type beats. According to ROC analysis, the RR and DI intervals were the best predictors of VF, with threshold values below 193 ms and 94 ms, respectively.

4.1.2. Absolute BVI parameters of the ECG intervals

The absolute BVI parameters were low during the pre-occlusion period but increased in both groups during ischemia. This increase was significantly greater in the 'VF+' group, particularly for parameters related to the QT and RT intervals (e.g., STV, RMSSD). These parameters exhibited excellent predictive value, with 93% sensitivity and 80% specificity. Instability indices of the RR interval also indicated VF risk, though they were less robust predictors compared to the repolarization parameters.

4.1.3. Equivalent analysis of ECG intervals selected for sinus rhythm

The sinus mean ECG intervals and the sinus BVI parameters sampled from the last arrhythmia-free period, either before VF in the ‘VF+’ group or before the 15th minute of ischemia in the ‘VF-’ group, notably revealed no significant differences between the ‘VF+’ and ‘VF-’ groups.

4.1.4. Frequency of arrhythmic beats and R on T arrhythmic beats

During the pre-ischemic and early ischemic phases, the number of arrhythmic beats was low and did not differ between the groups. However, in the pre-VF period, the frequency of arrhythmic beats and 'R-on-T' type beats was significantly higher in the 'VF+' group. Nonetheless, their predictive power was weaker compared to the repolarization-related BVI parameters.

4.1.5. Morphological characterization of arrhythmic beats

Analysis of the last 40 arrhythmic beats revealed no differences in morphological characteristics (e.g., R-wave amplitude, coupling intervals) between the 'VF+' and 'VF-' groups.

4.2. Self-controlled comparison of the absolute BVI parameters

To examine the changes in the absolute BVI parameters from baseline to VF occurrence, we conducted a self-controlled within-group comparison in the 22 control hearts that experienced VF during the entire 30-minute ischemic period. There was an immense increase in all absolute BVI parameters from the baseline (‘base’) to VF occurrence (‘30 min VF’).

5. DISCUSSION

According to our results, there was a strong relationship between instability of repolarization and VF occurrence in isolated rat hearts subjected to local ischemia. Absolute BVI parameters of ECG intervals that refer to repolarization had very high predictive power with high sensitivity and specificity for VF occurrence. In contrast, variability and instability parameters measured in sinus rhythm did not differentiate between the ‘VF+’ and ‘VF-’ groups of hearts and did not predict VF.

An increased number of preceding VPBs and ‘R on T’ VPBs were observed just before VF in hearts that subsequently experienced VF, but the characteristics of the preceding arrhythmic beats in terms of coupling interval and shape did not differ between the ‘VF+’ and ‘VF-’ groups. The increase in the absolute BVI parameters only partly resulted from the high number of arrhythmic beats, as the frequency of arrhythmic beats alone was a weaker predictor of VF than many absolute BVI parameters. The following sections consider the significance of the predictive properties of absolute BVI parameters.

5.1. Functional re-entry is the common mechanism of phase I VF and TdP; absolute BVI parameters quantify substrate instability contributing to functional re-entry

The absolute BVI parameters of ECG intervals quantify beat-to-beat electrical instability, which leads to spatial inhomogeneity—one of the key factors in functional re-entry. Previous studies have demonstrated that functional re-entry circuits sustain Torsades de Pointes (TdP) ventricular tachycardia, and re-entry-based excitation can also serve as a source for ischemia-induced VF.

The common substrate of TdP and phase I VF is inhomogeneity, arising from variations in repolarization due to different underlying mechanisms. In TdP, these variations are caused by genetic mutations, electrical remodeling, or ion channel dysfunction induced by drugs, whereas in ischemia, they result from regional differences (e.g., hypoxia, acidosis, potassium levels). In both cases, VF is most frequently triggered by ventricular premature beats (VPBs) occurring during the vulnerable period of repolarization.

The study's findings indicate that absolute BVI parameters related to repolarization predict phase I ischemia-induced VF with high sensitivity and specificity. The isolated Langendorff-perfused rat heart model effectively represents the mechanisms of ischemia-induced VF, and the results may also be relevant in other experimental or clinical contexts, although further studies are required to confirm this.

5.2. The predictive value and the role of arrhythmic beats in the development of ischaemic VF

Myocardial ischemia significantly increased the occurrence of arrhythmic beats and ‘R on T’ VPBs, with their frequency being higher in the ‘VF+’ group compared to the ‘VF-’ group. ‘R on T’ VPBs are considered dangerous as they can lead to fatal ventricular tachyarrhythmias and sudden cardiac death; however, they do not invariably result in VF. The increase in the frequency of arrhythmic beats alone was not sufficient to accurately predict VF, although it plays an important role in its development.

Arrhythmic beats can act not only as triggers but also enhance the heart's electrical inhomogeneity, which promotes the formation of re-entry mechanisms. Absolute BVI parameters, which measure beat-to-beat electrical instability, have much stronger predictive value for VF because they capture the cumulative effect of all factors contributing to

inhomogeneity. Our findings indicate that the presence of arrhythmias is critical in the development of both TdP and ischemia-induced VF, although the nature of the preceding arrhythmic activity differs between the two.

While in TdP, the variability of coupling intervals and the morphology of arrhythmic beats are the primary factors, in ischemia-induced VF, the increase in the frequency of arrhythmic beats is more determinant. The superior predictive capability of absolute BVI parameters arises from their ability to integrate all factors enhancing inhomogeneity into a single metric, as opposed to the isolated measurement of individual characteristics, such as beat frequency.

5.3. Cycle length variability

This study is the first to quantify repolarization instability preceding phase I ischemia-induced VF in isolated hearts. Traditionally, electrical instability is measured during sinus rhythm, with ectopic beats analyzed separately. The "absolute beat-to-beat variability parameters" developed in this study enable the combined assessment of these factors, introducing a novel perspective to the investigation of instability.

Previous research by Lemmert and colleagues demonstrated a strong association between RR interval instability and observations involving ventricular ectopic beats with the risk of ischemia-induced VF. Our findings support this, as STI, LTI, and TI parameters of the RR interval were significantly elevated before VF in isolated rat hearts. These irregularities, driven by ventricular arrhythmic activity, may facilitate the onset of VF.

It is important to distinguish between heart rate variability (HRV) and the absolute BVI parameters of RR intervals. While HRV focuses on sinus beats and is related to autonomic nervous system activity, absolute BVI parameters measure all beats, regardless of rhythm, including ectopic activity. Absolute BVI parameters serve as short-term predictors of ischemia-induced VF, in contrast to HRV, which acts as a long-term predictor in chronic heart disease. Thus, HRV and absolute BVI play distinct roles in assessing instability.

5.4. The variability of the repolarization measured regardless of the rhythm predict VF

The method we developed accounts for ventricular repolarization instability independently of rhythm regularity, which is critical for predicting arrhythmias such as VF.

Most existing approaches assess repolarization dynamics only during sinus rhythm or fixed-rate pacing, despite ectopic beats significantly increasing electrical instability. The instability of action potential duration caused by premature beats forms the basis for conduction blocks and re-entry circuits, which can lead to arrhythmias.

Chen and colleagues demonstrated that an increased frequency of VPBs and rhythm-independent measurements of QT instability predicted the onset of ventricular tachycardia (VT) in patients with acute myocardial infarction. Similarly, in our study, repolarization instability measured with absolute BVI parameters was the most reliable predictor of phase I ischemia-induced VF, while parameters measured during sinus rhythm were not predictive.

These findings suggest that evaluating repolarization instability independently of rhythm is essential for predicting ischemic arrhythmias, as it provides a more accurate assessment of ventricular electrical instability and the risk of VF development.

5.5. Analysis of ECG intervals during irregular rhythm elucidates the mechanism of phase I ischaemia-induced VF

Analysis of ECG intervals during irregular rhythms revealed that an increased frequency of arrhythmic beats and "R on T" VPBs, significant shortening of cycle length and electrical DIs, and an increase in the absolute BVI parameters of repolarization and cycle length predicted phase I ischemia-induced VF.

These findings detail the mechanisms of ischemia-induced VF and align with previous research. Laurita et al. demonstrated that VPBs increase the spatial inhomogeneity of repolarization. Ischemia induces significant repolarization variability in ischemic regions even in the absence of arrhythmic beats, while repolarization remains stable in non-ischemic myocardium. This disparity generates substantial spatial inhomogeneity between ischemic and non-ischemic regions, forming a re-entry substrate, with arrhythmic beats acting as triggers.

A high frequency of arrhythmic beats shortens cycle length, promoting the development of VPBs caused by delayed afterdepolarizations. The shortened cycle length leads to shorter DIs, and in combination with greater variability in repolarization duration, facilitates the initiation of re-entry.

6. CONCLUSIONS

The new *absolute* BVI parameters that predicted TdP risk in rabbits also predict VF risk during regional ischemia in rat hearts, showing a diagnostic and mechanistic similarity. Repolarization inhomogeneity seems crucial for ischemic VF induction, as absolute BVI parameters measuring repolarization variability showed excellent predictive power with high sensitivity and specificity. These newly validated biomarkers could act as substitutes for VF in preclinical drug studies.

7. LIMITATIONS

Given that repolarization in rat ventricles differs significantly from that in humans, these results may not be directly applicable to humans. Accurately predicting VF in the minute before its occurrence may not have clear clinical value. However, the absolute BVI parameters we have validated could serve as surrogates for VF in preclinical drug research, aligning with the 3Rs principle (refinement). Additionally, these validated biomarkers would be valuable in later translational research to identify potential benefits of new drugs against VF in a broader, low-risk human population.

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