Beat-to-Beat Repolarization Variability Measured by T Wave Spectral Variance Index in Chronic Infarcted Animals

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Background: Recent experimental and clinical studies have shown that beat-to-beat variability of repolarization morphology is associated with an increased risk for developing malignant ventricular arrhythmias. However, few data exist on the relationship between beat-to-beat repolarization variability and myocardial infarction itself.

Methods and Results: In the present work we tested the algorithm of T wave spectral variance (TWSV) using the two dimension fast Fourier transform, in an animal model of myocardial infarction to extend the evidences that support the existence of beat-to-beat alteration in repolarization during the chronic stage of myocardial infarction. Thirty-four New Zealand rabbits were included in the study and divided in two groups. Group I (N = 24) exposed to surgical ligation of the left anterior descendent coronary artery. Group II (N = 10) sham operated animals. The TWSV index was calculated before and after 15 and 45 days of surgery. Both groups showed significant increments in TWSV after 15 days postsurgery. However, while the sham animals return to its control value, the infarcted group exhibited values of the TWSV index that remains significantly high after 45 days of surgery, with a mean increment of 28.7% ($P < 0.05$ against sham). Moreover, when the infarcted group was qualitatively divided in three subgroups, according to its infarction areas, a trend was found in the correlation between the magnitude of the infarcted area and the TWSV index.

Conclusion: This noninvasive measure confirms the presence of temporal repolarization variability associated with chronic myocardial infarction and further contributes to identify the infarcted animals.

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Dispersion of ventricular repolarization is a measure of nonhomogeneous recovery of excitability during the repolarization process. This phenomenon is basically attributable to differences in action potential duration and activation times in different areas of the myocardium.^{1–3} Increments in the heterogeneity of repolarization are associated with an increased risk for the development of ventricular arrhythmias.^{4–9} Also, abnormalities in repolarization have been associated with an increasing risk to

sudden death.¹⁰ However, the ability to identify patients at risk with acceptable sensitivity and specificity remains limited. Several clinical tests to detect abnormalities during repolarization have been used to screen population for high-risk patients. Measurements of QT interval and its related dispersion calculated for a single beat from simultaneous leads, has been proposed as an indicator of spatial heterogeneity in repolarization. Another noninvasive approach to evaluate repolarization la-

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bility is to examine changes in repolarization pattern in a sequence of selected beats temporally distributed. There is growing evidence that changes in repolarization shape (more than in repolarization duration) contribute to increased heterogeneity of repolarization. In this regard, T wave alternans has been shown as an extreme manifestation of repolarization abnormality, and it has also been shown as highly related to an increased risk for ventricular tachyarrhythmias^{11,12}. Several methods have been proposed to characterize the temporal variability of repolarization morphology.¹³⁻¹⁵ However, one of the limitations associated with this kind of analysis rely in the exact identification of the T wave end points, which frequently fail in automatic analysis.

Therefore, in this experimental study we used the T wave spectral variance method, described by Steinbigler et al.,¹⁶ to test and quantify the presence of repolarization variability, in an animal model of chronic myocardial infarction. This technique allows us to detect dynamic changes in repolarization pattern either in amplitude or duration independently of the exact definition of the end of the T wave.

MATERIAL AND METHODS

Animal Model

Thirty-four New Zealand white male rabbits (3.2 to 3.8 kg) were anesthetized by intramuscular injection of a combination of ketamine (25 mg/kg) and xilacine (7 mg/kg). The animals were intubated, and manually ventilated with a combination of oxygen and air.

Using aseptic technique the hearts were exposed after removing the pericardium through a left intercostal thoracotomy. At this stage, animals were divided in two groups. The first group was composed of 24 animals in which the left anterior descending coronary artery was ligated with a silk suture, at the level of the first diagonal artery branch. Group 2 (sham) consisted of 10 animals in which the same surgical protocol was performed, except for the coronary ligation. The chest was then closed and the animals were allowed to recover. Heart rate and ECG were monitored in each rabbit for the first hour immediately following coronary occlusion, and arrhythmias appearing during this time were treated with intravenous lidocaine. All animals received routine postoperative care,

which included prophylactic administration of penicillin for the first 7 days.

ECG Measurements.

All animals were in sinus rhythm at the time of ECG recordings. ECG waveforms were recorded before surgery, and after 15 and 45 days of the postoperative period. A single precordial lead was located at 2 cm from the left sternal border in the fourth intercostal space. This lead normally exhibited a positive T wave with maximal deflection and was digitized at 1 kHz and 12-bit resolution using an analog-digital acquisition board (LabPC+, National Inst. Austin, TX, USA) to a Pentium computer. Data were processed using custom-built software made in Borland C++ 5.01 and running under Windows 98.

Approximately 400 beats were recorded from each animal, 50 Hz noise was digitally filtered if necessary. A template beat was manually selected, after this a fixed window of 200 ms, which included the complete T wave, was defined, as shown in Figure 1A. The remaining selected beats were aligned with a cross correlation algorithm using the peak of the R wave as the trigger point and the window predefined. All the ectopic or aberrant beats were automatically rejected by the computer. Baseline corrections were also performed if necessary by subtracting the PQ shift. This process finished with a matrix of 256 T waves temporally aligned as shown in Figure 1B. The T wave spectral variance was calculated with an algorithm described by Steinbigler et al.¹⁶ Briefly, the basements of the algorithm is the two-dimensional fast Fourier transform (2D-FFT). First, a one-dimensional fast Fourier transform is used and the frequency contents of the T waves matrix determined. The result is a one dimensional power spectrum for each T wave, in which the x axis corresponds to the frequency content in Hertz and the y axis to the magnitude of fast Fourier transform expressed in decibels.

The 2D-FFT is capable of evaluating the periodic appearance of the frequency content from a two-dimensional input matrix and the outcome is a two-dimensional power spectrum matrix. As shown in Figure 1C, the first dimension corresponds to the frequency contents of the signal expressed in Hz, and the second dimension reveals the periodicity of this frequency contents expressed in cycles per beat. A T wave spectral index

Figure 1. Consecutive T waves from 256 beats (Panel A) were acquired through a 200 ms. window to build the two-dimensional matrix shown in panel B. The T wave spectral variance index (TWSV) is calculated from the two-dimension fast Fourier transform (2D-FFT), (Panel C), applied to this matrix, dividing energies represented by beat-to-beat variation, (grey area) by total spectral energy (dark area + grey area). In the left side of the figure an schematic pattern of homogenous T waves is shown, the right panel in contrast shows a T wave with exaggerated beat-to-beat variability.

(TWSV) was calculated by dividing spectral energy with beat-to-beat variability greater to 0 cycles per beat by total spectral energy below 50 Hz (see Fig. 1).

A TWSV index near 0 is indicative of a constant T wave morphology in each of the 256 beats included for the analysis. In contrast, if different degrees of variability in the shape of the T wave are present, the TWSV index tend to 1, as it is schematically shown in Figure 1 (right panel). The frequency content of the T wave is less than 50 Hz, so

beat-to-beat variability appearing at frequency contents greater than this value, can be considered as noise.

Because it is difficult to measure the power level of noise between 0 and 50 Hz (T wave bandwidth), and considering that white noise has a flat frequency response at all spectral frequencies, an equivalent way to estimate the noise components is to measure all spectral energy found between 50 and 100 Hz (no spectral components of the T wave

appear at those frequencies and correspond to the same bandwidth of the T wave but shifted in the spectrum). The impact of noise in this technique was evaluated by Steimbigler et al.¹⁶ These authors have shown that the groups remain statistically different up to a noise/T wave amplitude ratio of 0.30. In our study, this ratio was less than 0.05 in all cases and no statistical differences between both groups of rabbits were found in the noise level.

Postmortem Evaluation of Infarct

After 45 days follow up the animals were sacrificed, the hearts were isolated and immersed in a formalin bath for posterior analysis. After removal of the auricles and atrioventricular valves, each heart was sliced into five transverse sections, which were approximately 3 mm thick, designated as A to E from base to apex. Histopathological analysis was performed in the superficial layers of the slices. The sections containing infarcted tissue were identified and the hearts were qualitatively classified, according to the extent of the infarcted area into three subgroups. The first subgroup was composed by 12 hearts showing evidence of transmural infarction, defined as homogeneous necrosis through the full thickness of the ventricular wall, located either in the slice B or slice C. The second subgroup was constituted by another 5 animals showing the area of infarction located proximal to the mesocardium, and the third subgroup consisted of 7 animals with no evidence of detectable macro infarction areas, by the methodological procedures used, (presumably only microfoci of infarction were present). None of the 24 animals exposed to coronary ligation exhibited evidence of infarction in slice A; this was probably due to the low level of the ligature.

Statistical Analysis

The results were presented as mean $\pm$ standard error of the mean (SEM). Comparison between means was performed by the Mann-Witney test in unrelated samples and the Wilcoxon sign rank test in related samples. A P value < 0.05 was considered significant.

RESULTS

The TWSV index, reflecting the temporal variability in the morphology of the T wave, was used

to quantify repolarization variability. A representative example is shown in Figure 2. The top panel contains the spectral 2D-FFT plots during control and after 45 days postsurgery for a sham animal. On the bottom panel, the same plots are shown for an infarcted rabbit. It can be clearly seen that in the sham operated animal the absence of changes in the TWSV index value together with the lack of changes in the 2D-FFT pattern are indicative of no changes in the beat-to-beat variability of the T wave. In contrast, the infarcted animals exhibit a 33.8% increment in the TWSV index and a different pattern of the 2D-FFT, demonstrating an increase in the temporal T wave variability.

Pooled data for both groups of animals are shown in Figure 3. It can be seen that the TWSV index significantly increased in both groups 15 days after surgery. However, after 45 days postsurgery, while the TWSV index returned to its control value (before surgery) in the sham group, it remained significantly high in the infarcted group. Moreover, the TWSV index of the infarcted group is also significantly different from that of the sham group at 45 days after surgery.

To evaluate the influence of the heart rate in T wave variability, we analyzed the R-R interval along the 256 beats considered for each experiment. Mean values of the R-R intervals for each group of animals were obtained by averaging the mean R-R interval of each experiment. After 45 days postsurgery no significant differences were found between the sham and the infarcted group (387 ± 31 ms versus 384 ± 83 ms, respectively).

We also evaluated the individual dispersion of the R-R interval by measuring the standard deviation of this variable for each animal. An average of 25 ± 11 ms was obtained for the sham group after 45 days postsurgery. This value was significantly different ($P < 0.05$) from that obtained in the infarcted group at the same time postsurgery (13 ± 12 ms). Moreover, no correlation was found between R-R interval variability and the TWSV index. In this regard the sham group exhibited a greater R-R interval variability and a smaller TWSV index than the infarcted group. This data contribute to demonstrate the lack of influence of the heart rate on repolarization variability in this experimental protocol.

Finally, the infarcted group of animals was qualitatively classified into three subgroups according to the extent of the scar area at 45 days after surgery. The corresponding TWSV indexes of each

Figure 2. Representative examples of a sham (top panel) and infarcted animal (bottom panel) before (left) and 45 days after surgery (right), are shown. The sham does not evidence any changes in the 2D-FFT pattern or TWSV index. On the contrary, the infarcted animal shows increments in the beat-to-beat variation of the spectral components, and a greater TWSV index.

subgroup were plotted together with those of the sham group as illustrated in Figure 4. A clear trend in the relationship between the extent of the infarcted area and the absolute values of the TWSV index can be appreciated.

Moreover, each of the three levels of infarction exhibited TWSV indexes significantly higher than that showed for the sham operated group.

DISCUSSION

Alterations in ventricular repolarization have been found to play an important role in arrhythmogenesis.¹⁻⁶ It was also suggested, that changes in the shape of the T wave, more than in duration exclusively, are associated with an increase in repolarization heterogeneity.¹⁰⁻¹⁵ The analysis of TWSV index developed by Steinbigler et al.¹⁶ is a novel noninvasive method for the evaluation of beat-to-beat variability in repolarization. The prin-

cipal advantage of this method relies on the absence of the necessity to detect the T wave endpoints, with the benefits that this implies due to the limited accuracy of automatic algorithms.¹⁷ Moreover this new analysis of repolarization variability based in 2D-FFT has been shown to successfully detect patients with myocardial infarct prone to ventricular fibrillation or tachycardia.

This experimental study extends the evidence that supports the existence of beat-to-beat alteration in repolarization during the chronic stage of myocardial infarction.

There was a high increment in TWSV index within the first 15 days after surgery in both groups, (sham and infarcted). This effect was probably produced by alterations associated with surgery. However, while the sham animals return to control values within 45 days after surgery, the infarcted group of animals exhibited a TWSV index

Figure 3. Pooled data showing TWSV index (mean $\pm$ SEM) for sham (open circles) and infarcted (solid circles) animals, calculated before and after 15 and 45 days of surgery. * $P < 0.005$ versus Sham (45 days); + $P < 0.05$, and ++ $P < 0.005$ versus before surgery.

that remained significantly high as compared with that obtained before surgery. The model of infarction implemented in the present study induced a lesion of variable dimensions located in the anterior wall of the left ventricle. This was probably due to interanimal variance in coronary collateral circulation. When the infarcted animals were classified according to the extension of the infarction, a trend in the correlation between the TWSV index and the infarcted area was also found, though no significant differences were seen among the three subgroups analyzed. Noninfarcted hearts exhibited the smallest TWSV index, and this magnitude increased associated with the particular pattern of the scar reaching a 34% mean increment in hearts with transmural infarction. On the other hand, significant differences were also found when the infarcted animals were compared against the sham group. These findings further suggest that repolarization abnormalities, in the form of subtle micro-volt level changes in T wave morphology, would be a marker of silent infarct or ischemia.

The existence of a necrotic area in the myocardium may seem to be the cause responsible for the increments found in the TWSV index. The mechanism by which the repolarization process is modified is difficult to assess in the present experimental conditions. However some hypothetical explanations could be offered. First, it is important to recognize that, in our experiments, the infarction has evolved to its chronic phase. These necrotic regions are electrically silent and could surround areas of intact tissue. Moreover, they could act like barriers to the activation or recovering process,

favoring division or fractionation of the wavefronts and leading to different patterns of activation and recovery in successive beats. Since the repolarization process propagates with a slow conduction velocity and induces low potential differences between adjacent regions, it could be more easily conditioned to the electrotonic influences of this isolated tissue. These possibilities might be a plausible explanation for the beat-to-beat changes in the T wave.

On the other hand, it has been well established that autonomic stimulation modulates the electrophysiological characteristics of myocardial cells. The presence of myocardial infarction disrupts neural transmission. In this regard, it has been postulated¹⁸ that transmural infarction could interrupt sympathetic and vagal transmission within the area of infarction, and produce sympathetic and vagal denervation at noninfarcted sites apical to the necrotic area. These denervated regions of normal tissue could produce action potentials that do not exhibit autonomic modulation as those from other areas which were normally innervated. This increment in action potential duration differences, could be translated to beat-to-beat variations in the morphology of the T wave.

Finally, the infarcted hearts exhibited a less R-R interval variability than normal hearts, suggesting that changes in the R-R interval may not play a significant role in determining the beat-to-beat variations of the T wave.

Figure 4. Bar graph of summary data showing the absolute value of TWSV index (mean $\pm$ SEM) of the sham group, and the infarcted animals subdivided in three levels according to the extent of the lesion. Differences among groups were significant. * $P < 0.05$ and ** $P < 0.005$ versus sham.

Limitations of the study

One of the principal limitations of the study is the lack of a quantitatively measurements of the infarcted area. An initial attempt was made to classify the different lesions based in microscopic cross section. Probably a different infarct animal model in which different size of infarction could be induced will be required to conclude that the TWSV index is correlated with infarct area.

Another limitation of this study was the use of only one precordial lead; probably it would be more adequate to use the magnitude vector derived from the orthogonal leads, especially in patients, to reduce the effects of changes in heart position and rotation.

Further studies such as larger clinical trials will be required to determine whether the TWSV index could be helpful in stratification of patients at risk for arrhythmic events and to better assess accuracy of this index in identifying patients with different degrees of infarction.

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